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Spring 2005

FROM THE DIRECTOR

H. Leon Thacker, DVM, PhD

It seems as though spring has finally arrived after what seemed to be a long winter. Thought in late March that the ground hog had seen his shadow twice back in February, but I guess the long winter makes the arrival of spring all the more welcome. The change of seasons has not slowed the work at ADDL; we are involved with the immunohistochemistry method testing of sheep and cervids for transmissible encephalopathy diseases. These tests are in support of surveillance programs, primarily of federal sponsorship. We are now testing all the samples collected in Indiana for scrapie of sheep and goats and for chronic wasting disease of deer and elk. We are also receiving many samples for Johne's disease testing in support of a federally assisted Indiana surveillance program. Many of you are, no doubt, involved with the collection of the Johne's disease test samples; it has been a struggle in the lab to accommodate the flooding batches of samples received. Our faculty and staff have done and continue to provide yeoman's contributions to supporting the testing of these programs in addition to the work of the day to day submissions of samples for diagnostic evaluation.

Our laboratory is now a member of the National Animal Health Laboratory Network which includes key diagnostic laboratories around the U.S. as support labs in early identification of animal diseases that may occur as a threat to our animal populations and food supply. On March 29, Dr. Randall Levings, who is Director of the National Veterinary Services Laboratory in Ames, Iowa, visited our lab to become better acquainted with our faculty and staff and answer queries regarding the federal labs and their interactions with state laboratories. We were happy to have Dr. Levings visit and appreciate the opportunity to strengthen our relationship with the national diagnostic laboratory system.

We continue to welcome your comments regarding our services. In the next issue of Diagnostic Forum, we will include a survey questionnaire and will appreciate your return of that questionnaire. It is our intent to serve you in the best way possible as the state supported, fully accredited, animal disease diagnostic laboratory of Indiana. Hope you have an enjoyable spring.

FINAL DIAGNOSIS:

Pituitary abscess syndrome in a sheep

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

History: A 7-8 year old, spayed Suffolk laboratory ewe was euthanized after presentation to the referring veterinarian with acute recumbency, fever, and dysphagia.

Gross Findings: The skin and auricular cartilage of the left pinna were markedly distorted by a large (~5x1.5 cm) abscess. The ipsilateral middle and inner ear were grossly unremarkable. Viscous green exudate was noted within the pituitary fossa, surrounding the pituitary gland and extending along the caudal fossa of the skull.

Histopathologic description: Extensive coagulative and liquefactive necrosis accompanied by variable neutrophilic infiltrate effaced predominantly the pars distalis of the pituitary gland. Numerous bacterial colonies were present within the affected areas. Additional microscopic alterations included mild chronic otitis externa with focal abscess formation in the left ear, and chronic suppurative perivasculitis affecting the carotid rete-cavernous sinus complex and the cerebral leptomeninges.

Comments: Gross histologic lesions of necrosuppurative hypophysitis (inflammation of the hypophysis or pituitary gland), with intralesional bacterial colonies, were consistent with those seen in pituitary abscess syndrome. This syndrome is an uncommon condition that has been described primarily in humans, cattle, goats and sheep, as well as rarely in horses. The condition is usually fatal. Although reports in the literature usually involve mature (> 2-year-old) animals, the condition has been observed in young cattle after application of nose rings or plastic nose devices to control suckling. Males, both intact and castrated, are apparently more at risk of developing the pituitary abscess syndrome. The aggressive head-butting behavior of bulls, rams, and bucks, leading to sinusitis or cranial trauma with secondary sepsis, might be a contributing factor. The common practice of ringing bull's noses can also be a source of sepsis. Cases are usually sporadic, and even during outbreaks, their occurrence is low (<2% of animals at risk).

Clinical signs may appear suddenly; the course ranges from one day to several weeks. Clinical presentations and histories are highly variable, precluding the description of a classic case. Nonetheless, common clinical findings in affected animals are depression, anorexia, head pressing, exophthalmos, abnormal stance and recumbency; the most common abnormalities noted at neurologic examination are those associated with cranial nerve functions, primarily with ocular function and dysphagia. Cranial nerve deficits are usually asymmetrical and progressive. It is believed that the pressure caused by the enlarging abscess and its extension laterally or dorsally results in damage to adjacent cranial nerves and their nuclei. Bradycardia might be evident, most likely due to compression of the hypothalamus followed by increased vagal tone. A history of infectious disease occurring one to three months before the onset of the neural signs might be reported.

Cerebrospinal fluid analysis is the most rewarding ancillary diagnostic aid. As in most bacterial diseases of the central nervous system, a predominantly neutrophilic pleocytosis with elevated total protein is evident. Definitive diagnosis is made at necropsy since the abscess is usually apparent on gross examination of the brain. In some cases, there is also the presence of osteomyelitis of the basisphenoid bone, single or multiple brain abscesses, and suppurative leptomeningitis at the ventral surface of the brain stem and cervical spinal cord.

The pathogenesis of the pituitary abscess syndrome is uncertain. Direct extension of an adjacent extracranial infection might be a possible cause of the disease. Possibilities include inner ear infection, sinusitis, tooth abscess, infection from a primary cranial fracture, ascending infection through a persistent craniopharyngeal duct and, in the case of horses, guttural pouch empyema. In this particular case, the isolation of identical bacterial organisms (namely *Arcanobacterium pyogenes* and *Escherichia coli*) from the ear abscess and the pituitary abscess suggests a causative relationship between these two lesions. The ipsilateral middle and inner ear were grossly unremarkable; the cause of the pituitary abscess by direct extension from the ear abscess cannot be completely ruled out, however, since the middle and inner ear were not further evaluated histologically.

The most likely routes by which bacterial organisms can reach the pituitary gland are arterial, venous, or lymphatic. In ruminants and pigs the gland is surrounded by a complex mesh of intertwined arteries and capillary beds known as the rostral epidural *rete mirabile* (carotid rete, see figure). This extensive capillary network makes the gland susceptible to bacterial seeding from other

chronic sources of infection such as mastitis, arthritis and lung abscesses or pneumonias.

A. pyogenes is the most common isolate from cattle, but a number of other Gram-positive (such as *Streptococcus* spp., *Staphylococcus* spp., and *Corynebacterium pseudotuberculosis)* and Gram-negative (*Fusobacterium necrophorum, Bacteroides* sp., *Pasteurella* spp., *Pseudomonas* spp., *Actinobacillus* spp.) organisms, as well as *Mycoplasma argini,* have reportedly been isolated in pure and mixed cultures from pituitary abscesses. These organisms are known to cause chronic suppurative conditions also in other organ systems. This further supports the concept of their circulatory spread.

In addition to dissemination through the blood vascular system, infections in the nasal chamber, paranasal sinuses and ear may spread to intracranial structures by way of the cavernous sinus, a valveless venous system that bathes the pituitary gland and connects with the veins of the extracranial soft tissues of the head. Alternatively, bacteria may gain access to the central nervous system and the pituitary gland through lymphatic channels that communicate with cerebrospinal fluid in the area of the nasal mucosa and the cribiform plate.

Clinically, the differential diagnoses for ruminants with the pituitary abscess syndrome should include listeriosis, bovine herpesvirus 5 infection, polioencephalomalacia, lead poisoning, other brain abscesses, and rabies. Thrombotic meningoencephalitis in cattle, parelaphostrongylosis and coenurosis in sheep and goats, and caprine arthritis encephalitis in goats should also be considered. In horses, differential diagnoses include bacterial meningitis, viral encephalitis (Eastern/Western equine encephalomyelitis, rabies), equine protozoal myelitis, trauma, leukoencephalomalacia, hepato-encephalopathy, and space-occupying masses (abscesses or neoplasia). Clinical presentation and cerebrospinal fluid analysis are usually helpful in differentiating these diseases from pituitary abscesses.

Although infrequently diagnosed, pituitary abscesses can cause signs in ruminants and less commonly in horses that mimic other infectious and toxic neurologic diseases. They should, therefore, be included in the differential diagnosis for central nervous system diseases in these species.

-by Dr. Ingeborg Langohr, ADDL Graduate Student

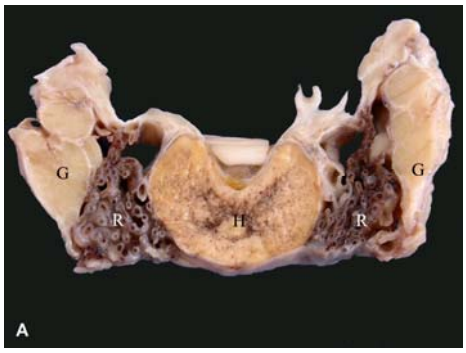

Figure A illustrates the intimate relationship between the ganglia of the fifth (trigeminal) cranial nerves (G), the carotid rete (R) and the pituitary gland (hypophysis, H).

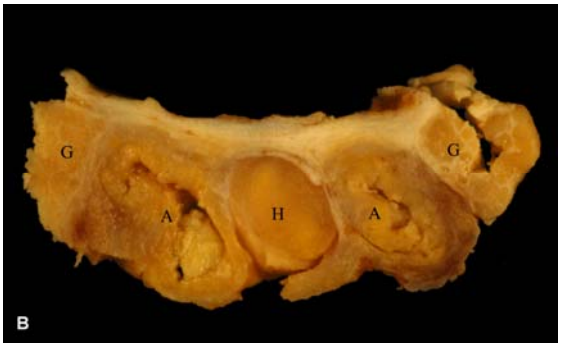

Figure B shows the same structures as in Figure A, including the pituitary gland (hypophysis H), and the trigeminal ganglia (G). Note the abscesses (A) effacing the carotid rete. Courtesy: Dr. Raquel Rech, Laboratory of Veterinary Pathology, Federal University of Santa Maria, RS, Brazil.

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Marek's Disease
(Visceral Leukosis)

Definition/Introduction: Marek's disease (MD) is one of the most common lymphoproliferative diseases of chickens which causes mononuclear infiltration of one or more of the following cells: peripheral nerves, gonad, iris, muscle, viscera, and skin. MD has been called by several names including "range paralysis", "neural lymphoma" and "skin leucosis".

Causative Organism: MD is caused by herpesvirus, which can be differentiated from other lymphoid neoplastic diseases. There are three serotypes of MDV which have many common antigens and are distinguished by serologic tests.

Serotype 1 Viruses	Serotype 2 Viruses	Serotype 3 Viruses(HVT)
Viruses grow best in duck embryo fibroblast or chicken kidney cell	Viruses grow best in chicken embryo fibroblast	Viruses grow best in chicken embryo fibroblast.
Virus grows slowly	Virus grows slowly	Virus grows rapidly.
Produce small plaques	Produce medium plaques	Produce large plaques

Jordan and Pattison, Poultry Diseases, 4th ed.

Virulence and oncogenicity are associated only with serotype 1 MDV's.

Pathogenesis: There are four phases of infection: 1) degenerative changes caused by early productive-restrictive virus infection, 2) latent infection, 3) another phase of cytolytic infection associated with permanent immunosuppression, and 4) nonproductive infected lymphoid cells that may or may not progress to lymphoma formation, a "proliferative" phase. The route of infection is inhalation. The virus then replicates in the lungs (in non-lymphoid cells). An acute phase of the disease can be seen within 72-96 hours where the lymphoid system, primarily bursa and thymus, undergoes cytolytic changes. Infected birds normally recover from the acute phase of the infection after 6-7 days and become latent. Infected lymphocytes carry the virus throughout the body, causing cell-associated viremia. Eventually, virus will be shed in the environment via feather debris and dander after the secondary cytolytic infection occurs in the feather follicle epithelium (~2 weeks post infection).

Transmission: MDV can be transmitted by direct and indirect contact between birds. Transmission is primarily by airborne route as the virus is shed in epithelial cells of the feather follicle, dander, chicken house dust, feces and saliva. The virus has a long survival time in dander since viable virus has been isolated from houses that have been depopulated for many months. (Historically, prior to vaccine availability, control in broilers was based upon early brooding exposure to used broiler litter and dander, marketing survivors versus poorer results with the thoroughly cleaned and disinfected brooder houses). Transmission by egg has no significance (i.e., chicken hatched and reared in isolation will be free of MDV.

Clinical signs: MD commonly affects pullets between 12-24 weeks of age, but can infect broilers as early as 6 weeks of age. The incubation period ranges from 3-4 weeks to several months. Signs may vary according to the nerve or nerves affected. Asymmetric progressive paralysis of one or more of the extremities can be seen. Wing involvement is characterized by drooping of the limb. Torticollis of nerves controlling the neck are affected. Vagal involvement will lead to dilatation of the crop and/or gasping. If the iris is involved, eyes will lose their ability to accommodate light intensity and blindness may occur (once called "grey eye"). Many birds die suddenly without symptoms. There are nonspecific signs such as weight loss, paleness, anorexia, and diarrhea.

Pathology: *Macroscopic lesions:* Nerve lesions can be seen as grayish, edematous, two or three times the normal thickness, and loss of the normal striated white glistening appearance. Nerves commonly affected include the brachial and sciatic plexi, celiac plexus, abdominal vagus and intercostals nerves. Nerve enlargement may not always be seen in affected birds at necropsy, although characteristic lesions may be found histologically. Also, tumors such as lymphoma occur in the ovary along with the nerve lesions. Macroscopic appearance in affected viscera, with the exception of the bursa of Fabricius, are indistinguishable from leukotic lesions induced by other agents (e.g. lymphoid leucosis virus). Organs are enlarged with diffuse grayish discoloration.

Microscopic lesions: There are two main types of lesions in peripheral nerves. Type A is interpreted as neoplastic in character, consisting of masses of proliferating lymphoblastic cells. Sometimes, demyelination and proliferation of Schwann cells are seen with these lesions. Type B is inflammatory in nature and is characterized by diffuse infiltration of lymphocytes and plasma cells, edema, and sometimes demyelination and Schwann cell proliferation. Lymphomatous lesions in visceral organs are more uniformly proliferative in nature. Deposition and diffuse proliferation of small to

medium lymphocytes, lymphoblasts, and primitive reticulum cells are seen. Plasma cells are rarely present.

Skin lesions are mostly inflammatory and can also be lymphomatous. Inflammatory cells are localized around the infected feather follicle. With small lesions, the integrity of the skin is maintained, but disruption of the epidermis leading to ulcer formation may occur with massive proliferation.

Herpesviruses replicate in the bursa of Fabricius and the thymus which results in degenerative changes in these organs. Atrophy of the thymus can be severe and involve the cortex and medulla. In some cases, lymphoid proliferation in the thymus was seen. Arterial lesions may occur in the aorta, coronary, celiac, gastric and mesenteric arteries which may show fatty proliferative changes.

Diagnosis: Since there is no truly pathognomonic gross lesions of MD and because MD lesions can closely resemble those of lymphoid leucosis (LL), the clinical diagnosis of MD has been considered difficult in practice. Infection of MDV, not necessarily accompanied by the clinical disease, can be detected by virus isolation or agar gel precipitation tests of viral antigen in feather tips or antibody in serum. These are useful features to differentiate Marek's disease from lymphoid leucosis.

Feature	Marek's disease	Lymphoid leucosis
Age	6 weeks or older	16 weeks or older
Symptoms	Frequently paralysis	Non-specific
Incidence	Frequently 5%+ in unvaccinated flocks	Rarely above 5%
Macroscopic Lesions -Neural enlargement -Bursa of Fabricius -Tumors in skin, muscle, proventriculus	 -Frequent -Diffuse enlargement or atrophy -May be present	 -Absent -Nodular tumors -Usually absent
Microscopic lesions **-**Neural involvement -Liver tumors -Spleen -Bursa of Fabricius CNS -Lymphoid proliferation of skin and feather follicles	 -Yes -Often perivascular -Diffuse -Either atrophy of follicles or interfollicular tumor -Yes -Yes	 -No -Focal or diffuse -Often focal -Intrafollicular tumors -No -No
Cytology of tumors	Pleomorphic lymphoid cells including lymphoblasts, small, medium and large lymphocytes and reticulum cells. Rarely may only be lymphoblasts	Lymphoblasts
Category of neoplastic lymphoid cell	T cell	B cell

(Jordan and Pattison, Poultry Diseases, 4th ed)

Treatment: There is no effective practical treatment for MD.

Prevention: *Vaccination:* Vaccines are extremely effective (90%+) in the prevention of Marek's disease. There are three serotypes: Serotype 1 which is available commercially as attenuated virulent or attenuated mildly virulent, Serotype 2 vaccines which are naturally non-pathogenic strains of MDV, or Serotype 3 "Herpes Virus Turkey (HVT) which are effective against virulent MDV but less effective against very virulent MDV. HVT was standard for the poultry industry throughout the 1970s, starting at over $.05/dose to as low as $3.00/1000 doses in the late 70s. It was developed at the Regional Poultry Research Lab in East Lansing, MI, now known as the Poultry Disease and Oncology Lab. Progress in the USA is due largely to USDA scientists.

Bivalent and Trivalent Vaccines: Synergistic effect and good protection can be achieved by combining the serotype vaccines 1,2, or 3 as bivalent or trivalent vaccines. These have become standard for the layer chick hatcheries, administered subcutaneously at hatching. Broiler chicks are given vaccine *in ovo* at the time of egg transfer.

Genetic selection: MD resistant chicks are obtained.

FAPP (filtered air positive pressure) ventilation: Biological filters to keep out airborne viruses are used.

For the commercial chick (layer and broiler) today, Marek's disease is not common due to vaccine use. For backyard operations, ease of vaccine handling and effective administration remains a challenge.

- by George Khalil, ECFVG Student
- edited by Dr. Tom Bryan, ADDL Poultry Diagnostician

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Purdue ADDL and Heeke ADDL will be closed on the following University holidays in 2005.

Memorial Day……..May 30
Independence Day……July 4
Labor Day……Sep 5
Thanksgiving……Nov 24-25
Christmas…… Dec 23,26

Purdue Animal Disease Diagnostic Laboratory West Lafayette, IN

Heeke Animal Disease Diagnostic Laboratory Dubois County, IN

Vesicular Stomatitis

Vesicular stomatitis is a reportable and zoonotic disease which is indistinguishable clinically from foot and mouth disease as well as other vesicular diseases. Unlike FMD, VS has a relatively low mortality, but does cause significant economic losses due to decreased weight gains, increased culling, reduced milk production, and increased labor costs in food animals as well as decreased performance, increased stall rest and increased labor costs in horses. The host range of VS is much more diversified than other vesicular diseases and includes susceptibility of horses, swine, cattle, llamas, sheep, goats, deer, bobcats, raccoons, monkeys and humans. Because of the implications to human health and the ramifications of suspected foot and mouth disease, it is imperative to report any suspected vesicular disease to the Indiana State Veterinarian.

Vesicular stomatitis is caused by an RNA virus within the family Rhabdoviridae. There are two serotypes: Indiana and New Jersey. The Indiana serotype has three subtypes: Indiana-1, Indiana-2, and Indiana-3. The serotypes have different epidemiologic and pathogenicity characteristics where the New Jersey serotype has a slightly higher attack rate and is more likely to cause hoof lesions. VS is easily inactivated by heat, UV light, and most disinfectants.

The clinical features of VS vary between species. The incubation period ranges from 1-5 days. In horses, cattle, llamas, and alpacas, the first signs of infection include ptyalism and fever. Vesicular lesions typically develop on the tongue and oral mucosa of these species and may progress to ulcers and erosions colonized by secondary bacterial infections. Swine usually exhibit lameness as the first sign of infection. Vesicular lesions in swine are generally located on the coronary band and snout. Cattle may additionally develop lesions around the coronary band, interdigital spaces, and teats although this is less commonly observed. Humans occasionally develop oral vesicles but, more commonly, develop flu-like symptoms including fever, chills, headache, muscle pain and runny nose.

Differential diagnoses for vesicular diseases include foot and mouth disease, swine vesicular disease, vesicular exanthema of swine, infectious bovine rhinotracheitis, bovine viral diarrhea and bluetongue. The following table helps explain the differences in the vesicular diseases.

Disease	Major Species Affected	Transmission
Foot and mouth	Swine, cattle, sheep, goats, African buffalos	Aerosol, contact, fomites
Swine vesicular disease	Swine, humans	Contact, fomites
Vesicular exanthema of swine	Swine, marine mammals	Contact, fomites
Vesicular stomatitis	Horses, cattle, swine, wildlife, llamas, alpacas, humans	Insects, direct contact

VS rarely causes death, but may be fatal due to secondary bacterial infections. Vesicles easily rupture so that ulceration and erosions are usually the only gross necropsy findings. Histologic findings may include epidermal edema, hyperplastic epidermis, reticular degeneration, and focal necrosis. VS virus may be seen by electron microscopy in vesicular fluids or fresh lesions.

The epidemiology of VS is not well understood. Evidence suggests that insect vectors carry the virus and are able to infect wildlife, domestic animals, and humans. VS can also be transmitted animal to animal and animal to human through direct contact. In the U.S., VS appears to be

enzootic in southern coastal plan states. The southwestern states have experienced an approximately 10 year interval of domestic animal VS outbreaks. The most recent outbreaks occurred in Colorado, New Mexico, and Texas in 1982-1983, 1995-1998 and 2004.

In conclusion, the OIE recognizes VS as a List A disease capable of "very serious and rapid spread, irrespective of national borders, that are of serious socio-economic or public health consequence and that are of major importance in the international trade of animals and animal products." Due to the recent southwestern outbreak in the U.S., it is important to check both state and international regulations regarding necessary testing and requirements for interstate and international shipment of livestock and horses.

-by Lyndsay Cross, Class of 2005
-edited by Dr. Leon Thacker, ADDL Director

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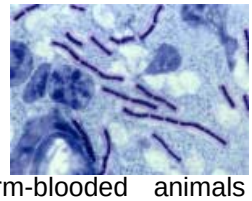
Retraction of Information

In the article titled "Mycoplasma in Cattle" published in the Fall 2004 issue of the ADDL Diagnostic Forum, it was stated that danofloxacin, enrofloxacin and tylosin are effective antibiotics for the treatment of Mycoplasma induced lameness of cattle. This was an editorial oversight that should not have been stated in the article. Danofloxacin is NOT approved for use in cattle.

Bacillus anthracis



Anthrax, caused by *Bacillus anthracis*, is an acute, febrile disease of virtually all warm-blooded animals including humans. It is most commonly characterized by septicemia and a rapidly fatal course.

Etiology and Epidemiology: The pathogen is present worldwide, usually in spore form. The soil is the main source of infection for herbivores. *B. anthracis* sporulates with greater frequency in low-lying marshy areas with soil rich in calcium and nitrate and a pH ranging from 5.0-8.0. Outbreaks are most often associated with neutral or alkaline, calcareous soils that serve as "incubator areas" for the organism. The spores apparently revert to the vegetative form and multiply to infectious levels in those areas where environmental conditions of soil, moisture, temperature, and nutrition are optimal.

Cattle, horses, mules, sheep and goats readily become infected when grazing in these areas, primarily in seasons when the minimal daily temperature is over 60°F (16°C). Epidemics tend to occur following climatic or ecological changes such as heavy rainfall, flooding, or drought; thus, it may occur irregularly, often with many years between occurrences.

Infection can occur from contaminated soil, water, bone meal, oil cake, tankage, offal, carrion birds, and wild animals. States within the United States where anthrax occurs include South Dakota, Nebraska, Arkansas, Texas, Mississippi, Louisiana and California; outbreaks and sporadic cases have, however, also occurred in other locations in the United States. Some regions of the Mississippi and Missouri River valleys harbor spores which are disseminated when flooding occurs.

In South Africa, non-biting blowflies may contaminate vegetation by depositing vomit droplets after feeding on a carcass infected with *B. anthracis.* This contamination is believed to be an important source of infection for browsing animals such as the kudu. Pigs, dogs, cats and wild animals can acquire the disease from consumption of contaminated meat.

Morphology: *B. anthracis* is a gram positive, non-motile, rectangular, aerobic, rod-shaped bacterium with square ends, measuring about 1µ x 3-5µ. Chain formation is common. After discharge from an infected animal, or when bacilli from an open carcass are exposed to free oxygen, spores are formed which are resistant to extremes of

temperature, chemical disinfectants, and desiccation. For this reason, the carcass of an animal that died from anthrax should not be necropsied.

Pathogenesis: In animals, the most common mode of infection is by ingestion. Infection may also take place via wounds, minor scratches and skin abrasions, and inhalation. Anthrax is not transmitted horizontally (from animal to animal or human to human).

The toxins and the capsule are the primary virulence factors of the anthrax bacillus. Virulent strains harbor two large plasmids: pX02 codes for the capsule and pX01 codes for the exotoxin. The anthrax toxin is complex, consisting of three protein components: I, II, and III. Component I is the edema factor (EF), component II is the protective factor (PA) and component III is the lethal factor (LF). Each component is a thermolabile protein. EF and LF gain entry into target cells by competitively binding with PA that has a membrane translocation function. These three components act synergistically to produce the toxic effects seen in anthrax. Components I and II cause edema with low mortality, but , when component III is included, there is maximum lethality. Only encapsulated, toxigenic strains are virulent.

Microorganisms in infected tissue exposed to air sporulate after several hours. After the spores enter the skin or mucosal membrane, they germinate at the site of entry. The vegetative cells multiply and are followed by the generation of edema, a papule in 12-36 hours, a vesicle, then a pustule, and finally a necrotic ulcer. From this lesion there is dissemination to the lymph nodes and finally the bloodstream, resulting in septicemia. Death is attributed to respiratory failure and anoxia caused by the toxin. Large numbers of bacilli are shed from the orifices during the terminal stage.

Clinical findings: *B. anthracis* is an obligate pathogen, the incubation period of which is 3-7 days (ranging from 1-14 days).

In herbivores, the clinical course ranges from peracute to chronic. The peracute form is characterized by sudden onset and a rapidly fatal course. Staggering, dyspnea, trembling, collapse, and a few convulsive movements may occur in cattle, sheep or goats without any previous evidence of illness.

In the acute form, there is an abrupt rise in body temperature and a period of excitement followed by depression, stupor, respiratory or cardiac distress, staggering, convulsions and death. The body temperature may rise to 107°F (41.5°C), animals may abort and rumination ceases. Bloody discharges from natural body orifices can appear.

Chronic infections are characterized by localized, subcutaneous, edematous swelling, most frequently at the area of the ventral neck, shoulders and thorax.

In horses, the disease is acute. Clinical signs may include pyrexia, chills, severe colic, anorexia, depression, weakness, bloody diarrhea, and swelling at the area of the neck, sternum, lower abdomen and external genitalia. Death usually occurs within 2-3 days of onset.

In pigs, the disease is usually subacute and may result in pharyngitis with extensive swelling and hemorrhages of the mouth and throat; however, an acute form may occur. An intestinal form with gastroenteritis also occurs, with non-specific clinical characteristics of anorexia, vomiting, diarrhea or constipation. Chronic infection with localization in the tonsils and lymph nodes of the cervical region is frequent.

In dogs and cats, the disease is rare and may resemble the clinical signs seen in pigs.

Humans develop localized cutaneous lesions called “malignant carbuncle” or “pustule” in more than 90% of cases. These are the result of contact of broken skin with infected blood or tissues. The site of infection in this form is most often the face, neck, hands, or arms. Humans can also acquire a highly fatal hemorrhagic mediastinitis (“woolsorter’s disease”) from spore inhalation when handling contaminated wool or hair. Following germination of spores, there is pulmonary necrosis, bacteremia and meningitis. Ingestion of undercooked meat contaminated with *B. anthracis* may lead to gastrointestinal anthrax. Mechanical transmission by blood-feeding insects was also reported, but is of minor importance.

Lesions: Rigor mortis is frequently absent or incomplete, and thick dark blood that fails to clot may ooze from body orifices. If the carcass is inadvertently opened, septicemic lesions are often observed. Hemorrhages frequently occur along the gastrointestinal tract mucosa and on the serosal surfaces of the thorax, abdomen, pericardium and endocardium. The spleen is typically enlarged, red-black and soft. The liver, kidneys, and lymph nodes are usually congested and enlarged.

Diagnosis: *Direct examination*: Smears from tissues or blood collected aseptically from superficial vessel and stained by Gram method. Polychrome methylene blue stain (M’ Fadyean’s stain) is another useful rapid presumptive diagnostic procedure (with this stain, rods appear blue surrounded by pink capsular material). One should keep in mind, however, that clostridial organisms are found in the blood shortly after death. They are

not square-ended, lack a capsule, and do not grow aerobically.

Isolation and cultivation: on blood agar plates and incubation at 37°C. Colonies appear within 24 hours. When virulent strains are grown in media containing serum or bicarbonate or both, they produce capsules and the colonies appear in 24 hours. They look flat, gray, are usually non-hemolytic, and smooth to mucoid. Some are called "medusa-head" or "judge's wig" type colonies since the edge of the colony resembles a tangled mass of curly hair. In the absence of serum or bicarbonate, bacteria fail to produce capsules and the colonies are rough.

Other methods of identification: Looking for string-of-pearls-like morphology (growth in the presence of penicillin creates chains of bacteria resembling a strong of pearls) and use of bacteriophage (a gamma phage added to a diffusely inoculated plate is expected to cause lysis only of *B. anthracis*.

Animal tests: used for confirmation of the diagnosis (*B. anthracis* is much more pathogenic for guinea pigs and mice than *B*. *cereus* and other *Bacillus* species, causing death within 24 hours. Large encapsulated rods are demonstrated in smears of spleen and blood of infected animals).

Immunity: Animals that recover from the infection have permanent immunity to the bacillus. Protective immunity is thought to be largely antitoxic and ELISA for PA, LF, and EF are used to confirm anthrax infection and monitor antibody responses.

Treatment: Sick animals should be separated and treated; all healthy animals should be immunized. The organism is susceptible to many antibiotics.

Immunization: Prevention of the disease is attained by annual vaccination of all grazing animals in the endemic area and by implementation of control measures during outbreaks. The Sterne's vaccine is approved for horses, cattle, sheep and pigs. It is used almost universally for livestock immunization. Vaccination should be done 2-4 weeks before the season when outbreaks may be expected. Animals should not be vaccinated within 2 months of anticipated slaughter. Since it is a live vaccine, antibiotics should not be administered within one week of vaccination.

A vaccine consisting of protective antigen from culture filtrate of an avirulent, non-capsulated strain has been used to protect U.S. military personnel and others at risk of infection. Multiple doses are given and an annual booster is required.

In addition to therapy and immunization, control of the disease in order to prevent its spread includes 1) notification of the appropriate regulatory offices, 2) rigid enforcement of quarantine, 3) prompt disposal of dead animals, manure, bedding and other contaminated material by cremation or deep burial, 4) isolation of sick animals and removal of healthy animals from the contaminated area, 5) disinfection of stables and equipment, and 6) improved sanitation

Bacterial and spore resistance: *B. anthracis* may survive for at least 2-3 decades in dried cultures. The microorganism remains viable in soil for many years. Freezing temperatures have little, if any, effect on the bacillus. Spores are destroyed, however, by boiling for 30 minutes and by exposure to dry heat at 140°F (60°C) for 3 hours. When used, most chemical disinfectants must be employed in high concentrations over a long period of time. Cremation or deep burial (at least 6 feet or 1.8m) in lime (calcium oxide) is recommended for disposal of the carcasses of animals that died of the disease.

Public Health Significance: Anthrax is seen most frequently in farmers, herdsmen, butchers, veterinarians, and in wool, tannery and slaughterhouse workers. Human infections most often result from spores entering injured skin, leading to cutaneous anthrax in more than 90% of cases. Pulmonary anthrax resulting from inhalation of spores is almost always fatal. Failure to diagnose human anthrax correctly and treat it adequately can result in death.

-by Inna Magner, ECFVG Student
-edited by Dr. Ingeborg Langohr, ADDL Graduate student

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4) Hungerford TG: 1990. Diseases of Livestock, 9th ed. McGraw-Hill, Sidney. Pp 329-332.

5) Pipkin AB: 2002. Anthrax. In Smith BP (ed) Large Animal Internal Medicine, 3rd ed. Mosby, St. Louis, MO. pp 1074-1076

Leon Thacker, ADDL Director, accepts the President's Award from IVMA President Dr. Janet Savill Sizemore at the Annual IVMA banquet, January, 2005.

Tom Hooper (top, left), Dr. Duane Murphy (middle,right) and Denise Riley (bottom, right) receive service awards from Purdue University. Tom has been at Heeke ADDL(located at the Southern Indiana Purdue Agriculture Center) for 20 years, Denise for 15 years, and Dr. Murphy for 10 years.

Also pictured is Dr. Tom Bryan, Heeke ADDL Avian Diagnostician.

Congratulations and thanks

ADDL NEWS

ADDL Serology technicians were 100% accurate on recent Pseudorabies check tests for serum neutralization, latex agglutination, ELISA, Particle Concentration Fluorescence Immunoassay (PCFIA) and IDEXX g1 procedures. In addition, they were 100% accurate on the 2004 Anaplasmosis cELISA proficiency test.

Bacteriology technicians were 100% accurate on the Johne's Disease Laboratory Approved Organism-based check test for bovine feces.

On the Road

Christina Wilson, Toxicology graduate student, chaired the Student Advisory Committee meeting at the annual Society of Toxicology meeting in New Orleans, March, 2005.

Dr. Zheko Kounev and Dr. Tom Bryan attended the Southeast Poultry Scientific Forum in Atlanta, Georgia, January, 2005.

Dr. Leon Thacker attended the annual Indiana Veterinary Medical Association meeting in Indianapolis, January, 2005.

Dr. Greg Stevenson attended the annual American Association of Swine Practitioners meeting in Toronto, Canada, March, 2005.

Paula Brost and **Deidre DuSold** attended the Indiana Society for Histotechnology meeting and workshops in Indianapolis, IN, March, 2005.

Dr. Leon Thacker chaired a meeting of the American Association of Veterinary Laboratory Diagnosticians Accreditation Committee and attended a Laboratory Quality Assessor's workshop in Las Vegas, NV, February, 2005.

Drs. Ching Ching Wu and **Tom Bryan** attended the North Central Avian Disease Conference/Midwest Poultry Federation in Minneapolis, MN, March, 2005