Winter 2003

FROM THE DIRECTOR

H. Leon Thacker, DVM, PhD

Winter has arrived. Even though up to now we have not had the severe weather that reached us 25 years ago in the form of the Blizzard of '78, this winter has been cold enough. The weather has been rough on animals and people. One bright spot, however, is that we have not had trouble with West Nile Virus lately.

The ADDL has recently been approved by site visit approval and passing of positive/negative check tests for running immunohistochemistry testing for transmissible encephalopathy diseases including Chronic Wasting Disease of deer and elk and Scrapie of sheep and goats. ADDL personnel participated with many members of the State Veterinarian's staff in collecting specimens from approximately 3300 hunter-shot deer from Indiana. The specimens will be used in testing for CWD to determine if the disease exists in the Indiana deer population. Although all tests have not yet been completed, testing to date for CWD in Indiana deer has been negative. Personnel of the Indiana Board of Animal Health are to be congratulated for coordinating a very efficient and scientifically guided effort in collecting specimens from each of Indiana's 92 counties.

Our laboratory has been performing thousands of tests on ear notch samples of feeder cattle looking for persistent infection of Bovine Virus Diarrhea. ADDL is using the fluorescent antibody test for these samples; check tests to date in comparing the FA to immunohistochemistry for persistent BVD infection have shown near complete agreement. In comparison testing, no false negatives have been found, results indicate that the FA may be even more sensitive.

Along with the ever advancing technology of communication, we have recently had practitioners send digital images of tumors before, during and after removal along with tissues for histopathologic evaluation so that the gross and microscopic appearance of the neoplasia can be compared. This provides for excellent collation of the gross and microscopic appearance and is excellent teaching material. We now have a digital microscope camera set in the lab so that histologic images of tumors can be returned also to practitioners.

We are attempting to get funding for two faculty positions in the West Lafayette laboratory restored in the upcoming state budget allocation. Any assistance that you may provide in replacing our virologist and one pathologist positions lost last year through budget cuts will be very much appreciated. Hope you have an uneventful and enjoyable winter; we are here to assist you with your diagnostic needs.

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FINAL DIAGNOSIS

Ataxia with Equine Herpes Virus Type 1 Infection in a Horse

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

History: A Thoroughbred mare, reportedly 17.5 years old, was submitted dead for necropsy to the Purdue Animal Disease Diagnostic Laboratory. The history included marked asymmetric ataxia for approximately four months. The animal did not respond to treatment for equine protozoal myeloencephalitis (EPM) and was euthanized. The owner reported herpes virus infection earlier in the year.

Gross Findings: Scattered petechial hemorrhages were seen in sections of spinal cord at the level of T17-L3, predominantly in the ventral and ventrolateral funiculi. The surrounding parenchyma was occasionally soft, with gray discoloration. The brain was grossly normal.

Histopathologic Findings: The main lesions in the spinal cord consisted of wallerian-like degeneration accompanied by non-suppurative myelitis with vasculitis. The degenerative lesions were present in all the sections examined, accompanied by minimal inflammatory lesions, with the caudal thoracic and cranial lumbar segments being the most severely affected, accompanied by multifocal hemorrhage. Multifocal non-suppurative encephalitis was observed in all sections of brain examined except the frontal cerebral cortex.

Discussion: The inflammatory reaction in sections of spinal cord from this animal was centered on blood vessels, occasionally accompanied by hemorrhage. These lesions are highly suggestive of a primary vasculitis with secondary ischemic injury to the neuroparenchyma. Immunohistochemistry revealed a positive staining for equine herpes virus-1 (EHV-1) antigen in smooth muscle cells of spinal arterioles, arteries, and veins. PCR for West Nile virus on a sample of spinal cord was negative. These results, as well as the reported previous infection by this virus, are highly suggestive of EHV-1 infection.

Herpes virus infection is ubiquitous in horses, and most animals are infected by the first year of life. Neurologic disease is an uncommon sequela to EHV-1 infection in horses and is usually, but not invariably, associated with a recent history of fever, abortion, or respiratory disease in the affected animal or herdmates. Myeloencephalopathy due to EHV-1 is commonly an epizootic disease, but may affect single animals in a herd. Pregnant or lactating mares may be more susceptible, although stallions, geldings, and foals have also been affected with this disease.

Natural EHV-1 infection is acquired via inhalation or ingestion of virus that is shed primarily from the nasopharynx. The incubation period for signs of neurologic disease after experimental and natural infection with EHV-1 is 6-10 days. This form of EHV-1 infection can occur at any time of the year, but the highest incidence is in the late winter, spring, and early summer, perhaps reflecting the seasonal occurrence of abortigenic EHV-1 infections during the same months. Neurologic signs are of abrupt onset, and horses may be found recumbent as the first evidence of the disease. Clinical signs most often reflect spinal cord lesions; ataxia and paresis of the pelvic limbs are common, and passive dribbling of urine is a characteristic feature. Signs may be mild and transient as recovery or compensation occurs with minimal lesions. With severe lesions, recumbency occurs in 12-24 hours from the onset of neurological signs. Sometimes ascending paralysis is observed and animals may die in coma or convulsion or be euthanized because of secondary complications. Morbidity rates ranging from less than 1% to almost 90% of exposed individuals and

mortality rates ranging from 0.5-43% of in-contact horses have been reported.

The pathogenesis involves viral endotheliotropism and associated vasculitis and thrombosis in the central nervous system with resultant ischemia and myelomalacia. Immune mechanisms have been implicated, but immunohistochemical demonstration of EHV-1 antigen in neurons of affected horses and association of myeloencephalopathy with certain EHV-1 strains indicate that primary viral neurotropism might also occur. EHV-1 has been reported to maintain a latent state in lymphoid tissues, leukocytes, and trigeminal ganglia. Reactivation of latent EHV-1 infection after stress situations has been reported as a possible source of infection.

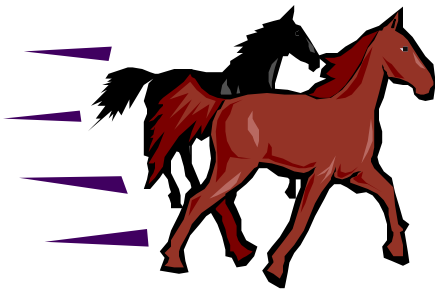
The cerebrospinal fluid from affected animals is often xanthochromic with elevated protein but normal numbers of cells (i.e., virtually none). During the early stage of the infection, there is a cell-associated viremia, and virus may be isolated from the buffy coat in affected horses; however, failure to isolate EHV-1 does not rule out the diagnosis. A 4-fold increase in serum neutralizing antibody titer is considered diagnostic for EHV-1 infection, and a single high titer (greater than 1:256) also suggests recent natural infection. PCR in whole blood, nasopharyngeal swabs, lymphoid tissues, and trigeminal ganglia is another valuable technique in supporting a diagnosis of EHV-1 myeloencephalopathy, but decreased or absent viral shedding due to stage of infection, previous vaccination, or viral latency might affect the viral DNA detection. Postmortem examination, including spinal cord histopathology and immunohistochemistry, are recommended to confirm the diagnosis of EHV-1 myelo-encephalopathy.

No specific treatment of equine herpes myeloencephalopathy is currently available; thus, management of horses with this condition is directed toward supportive nursing, nutritional care, and reduction of central nervous system inflammation. Treatment with the antiviral agent acyclovir has been recommended. Further studies are required, however, to evaluate the therapeutic efficacy of antiviral agents in the treatment of neurologic disease associated with EHV-1.

There is currently no known method to reliably prevent the neurologic form of EHV-1 infection. None of the EHV-1 or EHV-4 vaccines currently available carry a claim that they prevent EHV-1 myelo-encephalopathy, and the disease has been observed in horses vaccinated regularly at 3-4 month intervals with inactivated and modified live vaccines. Nevertheless, it is recommended to maintain appropriate vaccination procedures in an attempt to reduce the incidence of the other manifestations of EHV-1 infection and reduce the magnitude of challenge experienced by in-contact horses. This may indirectly help prevent EHV-1 myeloencephalopathy.

This case emphasizes that EHV-1 infection, although of rare occurrence, has to be considered as a possible cause for gait abnormalities affecting single or multiple horses on the premises, in addition to other viral myeloencephalitides, including West Nile virus, rabies virus, and togaviral encephalomyelitis, as well as equine protozoal myeloencephalitis, wobbler syndrome, cervical vertebral fracture, degenerative myelopathy, and a variety of plant and chemical intoxications.

-by Dr. Ingeborg Langohr, ADDL Graduate Student



Granulosa-Theca Cell Tumors in the Mare

Granulosa-theca cell tumors (GCT's) represent the most common group of tumors that develop in the equine ovary and probably comprise 2.5% of all equine tumors. These tumors have been reported in all ages and breeds, even in pregnant mares, but are most common in five- to ten-year-old mares. They arise from sex cord-stromal tissue within the ovary; most are benign and unilateral, but hormonally active. The opposite ovary is usually inactive, probably because inhibin produced by granulosa-theca cell tumors suppresses the pituitary follicle stimulating hormone (FSH) secretion.

Mares with GCT's usually exhibit one of three types of behavior depending upon the type and amount of hormones produced by their tumor. These are 1) prolonged anestrus, 2) persistent or intermittent estrus behavior (nymphomania), or 3) stallion-like behavior. Mares exhibiting the latter may also have a crested neck and enlarged clitoris.

Serum inhibin and testosterone are elevated in 87% and 54%, respectively, of mares with granulosa cell tumors. A serum testosterone concentration of >100 pg/ml is considered diagnostic for a GCT in a mare. Mares with elevated serum testosterone are those that usually exhibit stallion-like behavior. Other hormone levels, such as estrogen and progesterone, do not correlate to clinical signs.

The diagnosis of GCT's in mares is based on clinical history, including changes in behavior, rectal palpation, ultrasonography, and serum hormone analysis. By rectal palpation, the affected ovary is enlarged; it may be cystic and/or abnormally firm; an ovulation fossa is typically absent. Palpation of both ovaries is important because ovarian enlargement may be associated with other conditions, e.g. hematoma. If the contralateral ovary is active, the enlarged ovary probably does not have a GCT. A transitional ovary with multiple anovulatory follicles can be mistaken for an ovarian tumor. Sequential examination usually distinguishes between neoplastic and non-neoplastic conditions of the ovary, since tumors continue to enlarge.

Ultrasonography can provide additional diagnostic information, but may not provide a definitive diagnosis. Sonographic features of a GCT depend on the size and number of cysts within the tumor. Consequently, GCT's can be multilocular and honeycombed to dense, knobby or smooth. Some GCT's may appear with a single, fluid-filled cyst or as a solid ovarian mass. Ultrasonography of the contralateral ovary can demonstrate the presence or absence of follicle development and/or substantiate findings obtained by rectal palpation.

Grossly, GCT's are quite characteristic. The enlarged ovary is usually 10-20 cm in diameter, but may be 40 cm or larger. Loss of the ovulation fossa is due to the growth of the tumor, and usually occurs before the ovary enlarges. On cut surface, GCT's are polycystic, solid, or a combination of both. Cyst fluid is sanguinous or serous. Solid areas are white and grayish to yellow and orange, depending on the degree of hemorrhage that has occurred within the tumor. Larger tumors usually have areas of hemorrhage and/or necrosis. Histopathology generally provides a definitive diagnosis. Neoplastic granulosa-theca cells are generally oval to cuboidal and arranged in cords, trabeculae, sheets or tubules. In some tumors, neoplastic cells are arranged in rosettes resembling rudimentary follicles, so-called Call-Exner bodies, that may contain proteinaceous fluid.

Treatment for granulosa-theca cell tumors is surgical removal of the affected ovary. Most mares return to normal estrous cycles within 6-8 months following the ovariectomy, with a range of 2-16 months. Fertility usually returns to presurgical status after estrous cycles have been re-established.

-by Kristen Abderhalden-Telfer,
Class of 2002
-edited by Evan Janovitz, ADDL Pathologist

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

May 26, 2003..... Memorial Day
July 4, 2003.....Independence Day
September 1, 2003.....Labor Day

Our address has changed to

Animal Disease Diagnostic Laboratory
Purdue University
406 S. University St.
West Lafayette, IN 47907-2065

The Presence, Effect, and Diagnosis of Zearalenone in Dairy Cattle

Background

Mycotoxins are toxic substances that are produced by fungal species. These fungi can be found in a wide variety of plants and soil types. Toxigenic fungi are thought to be ubiquitous in the environment. Several characteristics are thought to be important for fungal growth, but the exact mechanisms of their proliferation are still unknown. The mycotoxins of major importance in Indiana are zearalenone, aflatoxin, DAS (vomitoxin) and fumonisin. Because the production of mycotoxins is strongly influenced by weather patterns and climates, the prevalence of each toxin varies with geographical location.

Zearalenone is naturally produced by the fungus *Fusarium roseum* and by some isolates of *Fusarium moniliforme*. Zearalenone is most commonly reported in the north central cornbelt of the United States and southeastern Canada. Zearalenone's estrogenic effects can be attributed to its complex chemical structure which makes it a phytoestrogenic molecule. Phytoestrogens are estrogenic compounds produced by plants/fungi. The fungus responsible for zearalenone production, *Fusarium spp*., has also been shown to produce the nonestrogenic toxins deoxynivalenol and T-2 under appropriate conditions. Therefore, veterinarians and producers should be aware that mycotoxin contamination can be a multi-factorial problem.

Clinical Effects

A wide variety of clinical effects attributed to zearalenone have been described in the literature. Decreased fertility, abnormal estrus cycles, swollen vulvas, vaginitis, reduced milk production and mammary gland enlargement are the most common findings reported in cattle and swine. From the aforementioned changes, single or multiple effects have been observed. A change in the estrus cycle can manifest itself

in various forms. Prolonged, skipped, or irregular heats are commonly associated with zearalenone effects. While these abnormal estrus changes are not exclusively specific to zearalenone toxicity, one should investigate feed related causes when increases in abnormal estrus cycles are observed on farm.

In one report from a 150 cow dairy herd, an increased artificial insemination index (decreased fertility) was reported after the herd had been fed moldy hay. Zearalenone concentrations in the hay extracts were reported to be 14mg/kg of hay. Upon removal of the infected hay, the AI index returned to the previous level. A 20 cow Brown Swiss herd was observed showing anestrus, false estrus, or nymphomania with a gray vaginal discharge. Infectious causes were ruled out, and feed samples were taken. Zearalenone concentrations of 50 ppb and 100 ppb were detected in the corn silage and haylage, respectively. Based on feed intakes, it was determined that these cows were receiving 1.6 mg of zearalenone per animal. Finally, an abnormal estrus cycle, nonresponsive to lutenizing hormone, was reported to have occurred in a dairy herd. Feed analysis yielded a zearalenone concentration of 25 mg/kg.

The clinical effects observed on heifers vary somewhat from cows. Mammary gland enlargement, swollen vulvas, and vaginitis are frequently observed more often in heifers as compared to cows. After being fed a ration containing moldy corn, 17 of 20 prepubertal dairy heifers developed enlarged mammary glands in at least one quarter. The secretion had a consistency of skim milk and appeared an off-white color. Zearalenone was determined to be present in the corn. Seven weeks after removal of the affected corn, all heifers were clinically normal. Weaver reported that when dietary zearalenone was greater than 12.5 ppm, a reduced conception rate was observed in virgin heifers.

Clinical effects of zearalenone vary by animal breed, age, and environment. While the majority of clinical symptoms observed mimic that of estrogenic stimulation, at the present time there is no way to determine what form of the syndrome will manifest in an affected group of animals. Multiple subclinical changes are probably occurring in affected animals. A combination of genetic and environmental factors most likely determines what outward clinical signs will be observed. Because the *Fusarium spp.* can produce estrogenic as well as nonestrogenic toxins, a variety of clinical signs are possible.

Feed Sampling and Diagnosis

Diagnosis can be quite difficult and frustrating to the veterinarian and owner. Animals may have consumed the offending agent prior to the veterinary investigation, thus making collection of diagnostic samples difficult or impossible. This problem is further exacerbated by the sporadic and variable nature of the toxin. Zearalenone, as with other mycotoxins, can only be detected in feed or feed products. No individual animal serologic or tissue test, antemortem or postmortem, exists at this time. Histopathological changes indicative of mycotoxicosis can be observed in individual animal tissues; however, this is a subjective assessment and does not support a definitive diagnosis. Clinical signs, such as vaginal discharge or vulva enlargement, can serve to further support zearalenone toxicity, but this too is a nonspecific indication. Sampling feed or feed products is the only diagnostic tool available.

In order to maximize the probability of isolating zearalenone or any mycotoxin, the investigating veterinarian should utilize several steps to aid in his/her efforts. *Feed samples taken should reflect the feed/forage utilized during the time period of the problem.* This is not always possible, especially if low-level contamination is present. Clinical signs indicating a problem may not appear until weeks after the infected feed was consumed. *Samples should be representative of the entire product being fed.* Zearalenone levels can vary dramatically from areas in the same storage unit, or even among kernels on the same ear of corn! Even if moldy appearing areas are present, sampling of normal

appearing feed (representative sample) should occur because normal appearing areas can be more severely affected. A good time to sample is after blending (such as auguring grain) has occurred. Periodic sampling of flowing grain/feed is recommended. Once individual stream samples have been collected, these can be combined and a subsample of at least 10 pounds should be submitted. *Samples should be submitted dry or frozen and protected from light.* Heat, chemicals, and sunlight all have the potential to alter mold metabolites from their original structure and activity. Once samples have been frozen or refrigerated they should remain that way. *Submit individual feeds rather than mixed feeds if at all possible.* Isolation of toxins in mixed feed is often difficult because of the complex nature of mixed feeds. If individual components are not available, a list of ingredients should be supplied to the diagnostic lab. As with any other diagnostic test, quality sampling and sample submission is of the utmost importance.

Several testing options are available for the diagnosis of zearalenone. Thin Layer Chromatography (TLC) is one of the earliest and simplest analytical methods that has been developed. Because TLC does not require expensive analytical machines and is relatively uncomplicated, most laboratories can utilize TLC as a diagnostic tool. The Purdue ADDL utilizes this method for determination of mycotoxins. The process of TLC consists of extracting solvents, sample clean-up/purification, solvents for separation, and detection methods. Because of the reliability, cost effectiveness, and quick results, TLC is utilized in many diagnostic laboratories A disadvantage of TLC is that the results are semi-quantitative.

High Performance Liquid Chromatography (HPLC) is another option for the diagnosis of mycotoxins. The sensitivity, accuracy, and quantification ability of this test has made HPLC popular in recent years. Cost of sampling is increased utilizing this method due to the expense of owning/operating the HPLC machine. HPLC samples also require extensive clean up, similar to TLC; however, an advantage is that quantitative, rather than semi-quantitative results, are obtained with HPLC.

Conclusion:

Dairy cow and heifer productivity can be greatly altered by the presence of zearalenone in feedstuffs. Diagnosing and isolating zearalenone is often a difficult and frustrating problem. A basic knowledge of fungal dynamics, proper sampling techniques, sample handling, and persistence are important aids in the diagnosis of mycotoxicosis. Several testing modalities are available at various diagnostic laboratories. Diagnosis of zearalenone-induced estrogenic effects is based on history, clinical signs, and detection of zearalenone in feed. Treatment is based on removal of the contaminated feed and replacement with high-quality feedstuffs. While the incidence of zearalenone toxicity varies greatly, producers and veterinarians should be aware of this estrogenic substance and its effects on dairy reproductive health.

-by Christopher Witte, Class of 2003
-edited by Dr. Steve Hooser, Chief of Toxicology, ADDL

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ADDL STAFF NEWS

In a ceremony at the ADDL on November, 5, 2002, **Dr. Phaedra Stiles**, ADDL graduate student, was commissioned a First Lieutenant in the Army Reserves by Captain Vivianna Mestas and Sergeant First Class Trevor Bethel. Dr. Stiles has been a member of the National Guard and, more recently, the Army Reserves, for the past 13 years.

ON THE ROAD

Dr. **Tsang Long Lin** presented his findings on Infectious Bursal Disease virus at the DNA Vaccine Conference in Edinburgh, Scotland, October, 2002.

Dr. **Ching Ching Wu** served as co-chair for the NC228 regional research meeting and co-authored two presentations at the Conference of Research Workers in Animal Diseases, St. Louis, Mo, November, 2002.

Dr. Randy White, Mary Woodruff,Virology lab supervisor and **Janeice Samman**, histology lab supervisor, attended a Chronic Wasting Disease Immunohistochemistry workshop at the National Veterinary Services Laboratory in Ames, Iowa, November, 2002.

Dr. **Steve Hooser** and **Christina Wilson** attended the Proteomics Symposium in Indianapolis, IN, November, 2002

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Toxoplasmosis in Small Animals

Toxoplasma gondii, an obligate intracellular coccidian protozoan parasite, is the cause of Toxoplasmosis and exposure to this organism is widespread among the human and domestic animal population (an estimated 30-40% of adults in the United States have antibodies to *T. gondii).*

Toxoplasma gondii is a tissue protozoan with three life stages – tachyzoites, bradyzoites and sporozoites. Tachyzoites is the rapidly dividing stage of the organism that disseminate in the blood or lymph during active infection and that can infect almost all tissues. The tachyzoites replicate intracellularly until the infected cell is destroyed, releasing the organism.

Clinical signs develop as a result of inflammation in infected tissues. If organism replication is attenuated by immune response, tissue cysts may develop that contain the more slowly dividing bradyzoites. Bradyzoites can later be reactivated under conditions of immunosuppression to divide rapidly as tachyzoites, potentially resulting in clinical disease. Bradyzoites can persist in affected tissues for the lifetime of the host.

Cats are the only species able to complete the coccidian life cycle of *T. gondii* in the intestinal tract. After cats ingest bradyzoites (that are encysted in tissues of prey animals) intestinal epithelial cells are infected and several rounds of asexual replication occur followed by the sexual cycle. Sporulated oocysts are passed in the feces; at this stage they are non-infectious. Sporozoites develop in the oocysts after one to five days of exposure to oxygen in conjunction with appropriate environmental temperatures and humidity.

Toxoplasmosis can be spread by ingestion of encysted bradyzoites in tissue, ingestion of food or water contaminated with feces that contain sporulated oocysts, or transplacental transmission. Cats are the key animal species in the epidemiology of *Toxoplasma gondii* because they are the only hosts that excrete the infective, environmentally resistant oocysts in their feces.

The intestinal schizogonous replication cycle in cats rarely causes clinical signs, but vomiting and diarrhea have been reported. In kittens, severe enteric disease can occur if concurrent disease is present (e.g., viral respiratory infection).

Clinical disease in the cat and other species is most commonly associated with the dissemination and replication of the organism during the extra intestinal cycle. Asexual reproduction occurs intracellularly in all body tissues except red blood cells and results in destruction of the infected cells plus subsequent clinical signs that vary depending on the organ systems most severely affected.

Tissue cysts rarely result in clinical signs but may serve as sources of antigen in immune-mediated diseases. Immuno-suppression can reactivate the bradyzoite cysts and lead to rapid dissemination, tachyzoite replication, and location-dependent clinical signs.

Respiratory tract involvement is common and is manifested by dyspnea and coughing. Anorexia, malaise, lameness, icterus, fever, tonsillar enlargement, lymphadenomegaly, splenomegaly and evidence of encephalitis are often observed depending on the site affected. Muscle discomfort from myositis is frequently noted during physical examination and neurological signs can be present.

Retinochoroiditis caused by organism replication is the primary ocular lesion, but other ocular manifestations include anterior and posterior chamber changes involving either one or both eyes. Secondary changes include vitreal hemorrhage, vitreal opacity, retinal detachment, iritis, iridocyclitis, hyphema, cataracts and corneal precipitates.

Diagnostic tests for toxoplasmosis include hematology, clinical chemistry, and urinalaysis. Although there are no laboratory findings pathognomonic for toxoplasmosis, suggestive clinical history and the following laboratory abnormalities raise the index of suspicion: non-regenerative anemia, neutrophilic

leukocytosis, monocytosis, eosinophilia, elevated creatine kinase, elevated alkaline phosphatase, elevated alanine transferase, elevated lipase, hyperbilirubinemia, hyperproteinemia, proteinuria and bilirubinuria.

Radiographic findings may include a diffuse interstitial to alveolar pattern with mottled lobar distribution in the thorax. In cats with CNS involvement, mass lesions may be detected by myelography, computed topography, or magnetic resonance imaging. Cytological examination may reveal tachyzoites in blood, CSF, transtracheal wash fluid, peritoneal effusion, or pleural effusion from clinically ill animals.

The short period of oocyst shedding, combined with the difficulty in demonstrating the oocysts, makes fecal examination a poor procedure for determining the status of *T. gondii* infection. However, due to the potential zoonotic risk, fecal examination should be performed in any cat with clinical signs suggestive of toxoplasmosis. When present, the oocysts are typically observed in the plane of view just beneath the coverslip. *T. gondii* oocysts are 10x12 µm in diameter and can be demonstrated microscopically in feline feces following flotation using solutions with specific gravity of 1.18 (sugar centrifugation is the preferred technique to demonstrate oocysts.)

Toxoplasma gondii specific antibodies and antigens can be detected using a range of tests that are commercially available including ELISA for both antibodies and antigens. Antigens are released intermittently from the tissues of cats up to one year post-infection. Because of the intermittent shedding, antigen detection cannot be used to differentiate infection from clinical disease or to predict oocyst shedding. Other commercially available techniques include immunofluorescent antibody assay, western blot immunoassay and the Sabin-Feldman dye test. ELISA, immunofluorescent antibody assay and western blot immunoassay have been adapted to detect IgM, IgG and IgA antibody responses. *T. gondii* DNA has been detected in the aqueous humor of cats with uveitis by polymerase chain reaction (PCR).

Tissue biopsy sections can be assessed for the presence of *T. gondii* by H&E staining or immunohistochemical staining. Immunohistochemical staining is superior to H&E staining because it is specific for *T. gondii*. It can be difficult to document the organism in the tissue of some clinically sick cats because of the small percentage of tissues evaluated histopathologically and because the pathogenesis of the disease in some cats may be immune-mediated.

Definitive diagnosis of clinical feline toxoplasmosis requires demonstration of the organism in the tissue in association with inflammation. This usually is achieved at necropsy in cats with overwhelming tachyzoite replication although a definitive diagnosis of clinical feline toxoplasmosis is occasionally made antemortem by demonstrating the bradyzoites and tachyzoites in tissue or effusions. Since *T. gondii*-specific antibodies can be detected in the serum, CSF and aqueous humor of normal as well as clinically affected animals, it is not possible to make a diagnosis of clinical toxoplasmosis based on those tests alone. However, a presumptive antemortem diagnosis of clinical feline toxoplasmosis may be used on the following combination of findings

- Demonstration of antibodies in serum, aqueous humor or CSF (documented exposure to *T. gondii*.)
- Demonstration of an IgM titer of above 1:64, a fourfold or greater increase in IgG titer
- Clinical signs disease referable to toxoplasmosis
- Exclusion of other common etiologies
- Positive response to appropriate treatment

Research is currently in progress to develop simple, inexpensive tests to detect active *T. gondii* infection. The proposed test would involve detection of two partially characterized secreted antigens called H4 Nd P18. Preliminary studies suggest that T. gondii tachyzoites actively secrete H4 and P18, suggesting that these antigens may circulate in the blood during acute infections. The antigens would be detectable by a simple antigen-capture blood test. This study subsequently could lead to the development of a commercially available, rapid test for diagnosis of acute toxoplasmosis of cats.

-by Roman Arteaga, ECFVG Student
-edited by Dr. Theresa Boulineau, ADDL Graduate Student

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Dr. Roland Winterfield (left), retired ADDL avian diagnostician is presented a plaque by Dr. Leon Thacker, ADDL Director, naming him an honorary diplomate of the American College of Poultry Veteri-narians. Dr. Winterfield was on faculty at the ADDL from 1966-1985.

In November, 2002, veterinarians from the Indiana State Veterinarians office and the USDA used ADDL facilities to take samples from nearly 3000 hunter-killed deer as part of a Chronic Wasting Disease monitoring program.

Percent of Microorganisms Resistant to Selected Antibiotics for 2002

Antibiotic	Canine										Equine												Feline							
	E. Coli		Enterococcus sp.		Pse. aeruginosa		Staph. aureus		Staph. intermedius		E. Coli		Salmonella sp.		Staph. aureus		Staph. epidermidis		Strep. equi		Strep. zooepidemicus		E. Coli		Enterococcus sp.		Pse. 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	1	18	38	0	2	0	4	0	0	0	0	0	0	0	0	0	0	71	100	65	100	0	0	0	71	0	0	0	0
Amoxycillin/Clauvulinic acid	24	18	9	19	93	95	31	4	0	2	8	27	25	20	43	50	38	0	0	0	0	0	11	14	33	43	100	100	0	0
Ampicillin	48	48	9	23	100	95	54	46	44	42	36	50	25	40	71	50	38	50	0	0	0	5	44	38	33	43	100	100	100	33
Cefazolin	23	18	64	62	100	98	23	4	0	0	12	27	25	20	43	50	25	0	0	0	0	5	0	10	100	100	100	100	0	0
Cefotaxime	13	7	9	33	7	10	23	4	0	0	0	0	0	0	43	50	25	0	0	0	0	5	0	5	33	57	0	20	0	0
Cefoxitin	24	17	91	81	100	95	23	4	0	0	8	19	25	20	43	50	25	0	0	0	0	5	0	0	67	86	100	100	0	0
Ceftiofur	20	16	73	82	100	98	23	4	0	0	0	12	13	20	57	50	25	0	0	0	0	5	0	5	100	100	100	80	0	0
Cephalothin	17	23	36	57	100	95	23	4	0	0	12	31	25	20	43	50	25	0	0	0	0	5	22	19	67	86	100	100	0	0
Chloramphenicol	15	16	0	0	67	80	0	0	3	0	4	38	13	40	0	0	13	0	0	0	0	0	0	5	33	0	67	60	0	0
Ciprofloxacin	16	16	0	10	13	17	15	8	3	2	4	4	0	0	14	0	13	0	0	0	0	0	6	10	0	43	0	0	0	67
Clindamycin	99	100	82	86	100	95	0	8	15	9	100	100	100	100	0	0	0	0	14	0	0	5	100	100	100	86	100	100	0	0
Enrofloxacin	16	18	27	45	27	55	15	17	3	2	4	4	0	0	14	0	13	0	0	0	0	0	6	14	33	57	0	40	50	67
Erythromycin	99	100	0	23	100	95	8	17	15	9	100	100	100	100	29	50	0	50	14	0	0	5	100	100	67	43	100	100	0	0
Gentamicin 500 microgm/ml	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14	0	0	50	0
Gentamicin	9	11	9	32	0	10	8	1	3	0	16	31	0	0	14	50	0	0	0	100	20	55	0	19	67	57	0	0	0	0
Sulphadimethoxine/Ormetoprim	29	29	0	10	100	95	8	13	15	9	40	62	0	40	43	50	13	50	0	0	0	5	11	19	0	14	100	100	0	0
Oxacillin + 2% NaCl	99	100	91	76	93	95	23	4	0	0	100	100	100	100	43	50	0	0	0	0	0	0	100	100	100	86	100	100	0	0
Penicillin	99	100	9	27	100	98	46	42	44	38	100	100	100	100	71	50	25	50	0	0	0	5	100	100	33	43	100	100	100	33
Rifampin	97	97	36	33	100	95	0	0	0	0	100	100	100	100	0	0	25	0	0	0	0	0	94	100	33	29	100	100	0	0
Tetracycline	31	33	36	52	33	27	8	17	12	16	36	42	13	20	29	50	0	50	0	0	30	50	17	24	100	86	33	40	50	0
Ticarcillin	43	42	18	24	13	7	46	42	44	38	32	42	13	20	71	50	38	50	0	0	0	5	33	29	33	71	0	40	100	33
Tribrissen	29	29	0	10	33	29	15	42	32	47	40	62	0	40	43	50	25	50	0	0	0	9	11	14	0	14	33	40	0	0
Vancomycin	99	100	0	10	100	98	0	0	3	0	100	100	100	100	0	0	13	0	0	0	0	0	100	100	0	0	100	100	0	0
Number of isolates	74	90	11	21	15	41	13	24	33	45	25	26	8	5	7	2	7	2	7	1	20	22	18	21	3	7	3	5	3	3

Percent of Micro-organisms that are Resistence to Selected Antibiotics for 2002

Antibiotic	Beef								Dairy						Swine							
	E. coli		Past. Haemolitica		Past. Multocida		Salmonella sp.		E. coli		Staph. aureus		Salmonella sp.		APP		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.
Ampicillin	49	46	0	0	0	0	29	43	56	62	63	17	66	80	15	6	64	64	53	41	1	3
Apramycin	7	12	20	22	40	67	0	29	39	35	na	na	21	26	2	13	21	20	10	9	33	41
Ceftiofur	20	19	0	0	0	0	29	43	25	26	4	0	45	50	2	0	8	10	8	6	7	8
Chlortetracycline	74	69	20	0	0	0	57	43	74	91	na	na	66	85	11	10	99	86	84	76	90	96
Clindamycin	100	100	100	100	100	100	100	100	94	97	na	na	97	100	8	19	99	100	100	100	89	82
Enrofloxacin	14	12	0	0	0	0	0	0	20	12	na	na	0	0	2	0	0	0	0	0	2	3
Erythromycin	100	100	20	0	60	0	100	100	95	98	na	0	97	100	0	3	99	99	100	100	91	80
Florphenicol	100	100	20	11	60	0	100	100	94	98	na	na	97	98	2	0	99	95	96	97	68	67
Gentamicin	19	27	20	0	60	0	0	29	53	59	na	na	24	35	2	0	19	21	10	15	2	5
Neomycin	31	42	80	44	80	67	43	43	65	71	na	na	59	67	9	3	46	43	24	15	60	63
Oxytetracycline	76	69	40	33	60	50	57	43	74	92	na	na	66	85	32	35	99	88	84	76	92	97
Penicillin	100	100	20	44	0	17	100	100	95	98	33	17	97	100	72	90	99	100	100	100	8	12
Sulphadimethoxine	69	58	60	44	60	83	57	57	64	73	70	92	76	83	15	10	74	71	66	71	63	62
Spectinomycin	36	38	40	78	80	50	86	100	63	76	na	na	66	93	13	0	65	55	90	100	19	29
Sulphachloropyridazine	69	58	20	11	80	83	57	43	75	89	na	na	79	83	25	35	74	72	64	68	66	60
Sulphathiazole	69	58	80	56	80	83	57	43	76	89	na	na	79	83	21	23	75	73	64	68	67	65
Tiamulin	100	100	100	78	60	83	100	100	93	98	na	na	97	100	15	0	99	100	100	100	22	25
Tilmicosin	98	100	0	0	60	0	100	100	92	96	na	na	97	100	4	0	98	98	100	100	88	77
Triple Sulfa	38	42	20	0	0	0	0	43	57	61	na	na	28	37	2	0	14	18	12	12	3	6
Tylosin	100	100	100	89	60	83	100	100	94	98	na	na	97	100	na	na	99	100	100	100	na	na
number of isolates	67	26	5	9	5	6	7	7	112	85	27	12	26	46	53	31	188	96	51	34	123	117

Jan. 1 - June 30, 2002

Antibiotic Resistence (%)	Canine					Equine						Feline			
	E. Coli	Enterococcus sp.	Pse. aeruginosa	Staph. aureus	Staph. intermedius	E. Coli	Salmonella sp.	Staph. aureus	Staph. epidermidis	Strep. equi	Strep. zooepidemicus	E. Coli	Enterococcus sp.	Pse. aeruginosa	Staph. aureus
Amikacin	1	18	0	0	0	0	0	0	0	71	65	0	0	0	0
Amoxycillin/Clauvulinic acid	24	9	93	31	0	8	25	43	38	0	0	11	33	100	0
Ampicillin	48	9	100	54	44	36	25	71	38	0	0	44	33	100	100
Cefazolin	23	64	100	23	0	12	25	43	25	0	0	0	100	100	0
Cefotaxime	13	9	7	23	0	0	0	43	25	0	0	0	33	0	0
Cefoxitin	24	91	100	23	0	8	25	43	25	0	0	0	67	100	0
Ceftiofur	20	73	100	23	0	0	13	57	25	0	0	0	100	100	0
Cephalothin	17	36	100	23	0	12	25	43	25	0	0	22	67	100	0
Chloramphenicol	15	0	67	0	3	4	13	0	13	0	0	0	33	67	0
Ciprofloxacin	16	0	13	15	3	4	0	14	13	0	0	6	0	0	0
Clindamycin	99	82	100	0	15	100	100	0	0	14	0	100	100	100	0
Enrofloxacin	16	27	27	15	3	4	0	14	13	0	0	6	33	0	50
Erythromycin	99	0	100	8	15	100	100	29	0	14	0	100	67	100	0
Gentamicin 500 microgm/ml	0	0	0	0	0	0	0	0	0	0	0	0	0	0	50
Gentamicin	9	9	0	8	3	16	0	14	0	0	20	0	67	0	0
Sulphadimethoxine/Ormetoprim	29	0	100	8	15	40	0	43	13	0	0	11	0	100	0
Oxacillin + 2% NaCl	99	91	93	23	0	100	100	43	0	0	0	100	100	100	0
Penicillin	99	9	100	46	44	100	100	71	25	0	0	100	33	100	100
Rifampin	97	36	100	0	0	100	100	0	25	0	0	94	33	100	0
Tetracycline	31	36	33	8	12	36	13	29	0	0	30	17	100	33	50
Ticarcillin	43	18	13	46	44	32	13	71	38	0	0	33	33	0	100
Tribrissen	29	0	33	15	32	40	0	43	25	0	0	11	0	33	0
Vancomycin	99	0	100	0	3	100	100	0	13	0	0	100	0	100	0
number of isolets	74	11	15	13	33	25	8	7	7	7	20	18	3	3	3

Antibiotic Resistence (%)	E. Coli	Past. Haemolitica	Past. Multocida	Salmonella sp.	E. Coli	Staph. aureus	Salmonella sp.	APP	E. coli	Salmonella sp.	Strep. suis 1-10
Ampicillin	49	0	0	29	56	63	66	15	64	53	1
Apramycin	7	20	40		39	na	21	2	21	10	33
Ceftiofur	20	0	0	29	25	4	45	2	8	8	7
Chlortetracycline	74	20	0	57	74	na	66	11	99	84	90
Clindamycin	100	100	100	100	94	na	97	8	99	100	89
Enrofloxacin	14	0	0	0	20	na	0	2	0	0	2
Erythromycin	100	20	60	100	95	na	97	0	99	100	91
Florphenicol	100	20	60	100	94	na	97	2	99	96	68
Gentamicin	19	20	60	0	53	na	24	2	19	10	2
Neomycin	31	80	80	43	65	na	59	9	46	24	60
Oxytetracycline	76	40	60	57	74	na	66	32	99	84	92
Penicillin	100	20	0	100	95	33	97	72	99	100	8
Sulphadimethoxine	69	60	60	57	64	70	76	15	74	66	63
Spectinomycin	36	40	80	86	63	na	66	13	65	90	19
Sulphachloropyridazine	69	20	80	57	75	na	79	25	74	64	66
Sulphathiazole	69	80	80	57	76	na	79	21	75	64	67
Tiamulin	100	100	60	100	93	na	97	15	99	100	22
Tilmicosin	98	0	60	100	92	na	97	4	98	100	88
Triple Sulfa	38	20	0	0	57	na	28	2	14	12	3
Tylosin	100	100	60	100	94	na	97	na	99	100	na
number of isolates	67	5	5	7	112	27	26	53	188	51	123

Antibiotic Resistence (%)	Canine					Equine						Feline			
	E. Coli	Enterococcus sp.	Pse. aeruginosa	Staph. aureus	Staph. intermedius	E. Coli	Salmonella sp.	Staph. aureus	Staph. epidermidis	Strep. equi	Strep. zooepidemicus	E. Coli	Enterococcus sp.	Pse. aeruginosa	Staph. aureus
Amikacin	1	38	2	4	0	0	0	0	0	100	100	0	71	0	0
Amoxycillin/Clauvulinic acid	18	19	95	4	2	27	20	50	0	0	0	14	43	100	0
Ampicillin	48	23	95	46	42	50	40	50	50	0	5	38	43	100	33
Cefazolin	18	62	98	4	0	27	20	50	0	0	5	10	100	100	0
Cefotaxime	7	33	10	4	0	0	0	50	0	0	5	5	57	20	0
Cefoxitin	17	81	95	4	0	19	20	50	0	0	5	0	86	100	0
Ceftiofur	16	82	98	4	0	12	20	50	0	0	5	5	100	80	0
Cephalothin	23	57	95	4	0	31	20	50	0	0	5	19	86	100	0
Chloramphenicol	16	0	80	0	0	38	40	0	0	0	0	5	0	60	0
Ciprofloxacin	16	10	17	8	2	4	0	0	0	0	0	10	43	0	67
Clindamycin	100	86	95	8	9	100	100	0	0	0	5	100	86	100	0
Enrofloxacin	18	45	55	17	2	4	0	0	0	0	0	14	57	40	67
Erythromycin	100	23	95	17	9	100	100	50	50	0	5	100	43	100	0
Gentamicin 500 microgm/ml	1	0	0	0	0	0	0	0	0	0	0	0	14	0	0
Gentamicin	11	32	10	1	0	31	0	50	0	100	55	19	57	0	0
Sulphadimethoxine/Ormetoprim	29	10	95	13	9	62	40	50	50	0	5	19	14	100	0
Oxacillin + 2% NaCl	100	76	95	4	0	100	100	50	0	0	0	100	86	100	0
Penicillin	100	27	98	42	38	100	100	50	50	0	5	100	43	100	33
Rifampin	97	33	95	0	0	100	100	0	0	0	0	100	29	100	0
Tetracycline	33	52	27	17	16	42	20	50	50	0	50	24	86	40	0
Ticarcillin	42	24	7	42	38	42	20	50	50	0	5	29	71	40	33
Tribrissen	29	10	29	42	47	62	40	50	50	0	9	14	14	40	0
Vancomycin	100	10	98	0	0	100	100	0	0	0	0	100	0	100	0
number of isolets	90	21	41	24	45	26	5	2	2	1	22	21	7	5	3

Jul. 1 - Dec. 31, 2002	Beef				Dairy			Swine			
Antibiotic Resistence (%)	E. Coli	Past. Haemolitica	Past. Multocida	Salmonella sp.	E. Coli	Staph. aureus	Salmonella sp.	APP	E. coli	Salmonella sp.	Strep. suis
Ampicillin	46	0	0	43	62	17	80	6	64	41	3
Apramycin	12	22	67	29	35	na	26	13	20	9	41
Ceftiofur	19	0	0	43	26	0	50	0	10	6	8
Chlortetracycline	69	0	0	43	91	na	85	10	86	76	96
Clindamycin	100	100	100	100	97	na	100	19	100	100	82
Enrofloxacin	12	0	0	0	12	na	0	0	0	0	3
Erythromycin	100	0	0	100	98	0	100	3	99	100	80
Florphenicol	100	11	0	100	98	na	98	0	95	97	67
Gentamicin	27	0	0	29	59	na	35	0	21	15	5
Neomycin	42	44	67	43	71	na	67	3	43	15	63
Oxytetracycline	69	33	50	43	92	na	85	35	88	76	97
Penicillin	100	44	17	100	98	17	100	90	100	100	12
Sulphadimethoxine	58	44	83	57	73	92	83	10	71	71	62
Spectinomycin	38	78	50	100	76	na	93	0	55	100	29
Sulphachloropyridazine	58	11	83	43	89	na	83	35	72	68	60
Sulphathiazole	58	56	83	43	89	na	83	23	73	68	65
Tiamulin	100	78	83	100	98	na	100	0	100	100	25
Tilmicosin	100	0	0	100	96	na	100	0	98	100	77
Triple Sulfa	42	0	0	43	61	na	37	0	18	12	6
Tylosin	100	89	83	100	98	na	100	na	100	100	na
number of isolates	26	9	6	7	85	12	46	31	96	34	117