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Summer 2000

FROM THE DIRECTOR

H. Leon Thacker, DVM, PhD

This is another great time of year in Indiana. As our seasons change, we have the opportunity to look forward to the enjoyments that each of the seasons bring us; we can now look forward to lowered humidity, longer and cooler nights, the pleasures that come along with Fall activities and those many blessings that we so often take for granted. The activities of the season change have set in also at ADDL; the undergraduate students are back on campus, Purdue's Fall Vet Conference is just around the corner, the rush for testing for fairs and sales is over, and we can start looking for a new group of diseases that affect our animal populations with the climate changes.

We have had some administrative changes in the ADDL, along with some limited personnel changes. On July 1, 2000, Dr. Bill Van Alstine stepped down as assistant director of the Laboratory and Dr. Steve Hooser has stepped into the position. Thanks are herein expressed to Dr. Van Alstine for the past 18 months he spent as assistant director ; his input was much appreciated. Bill will be taking on more diagnostic and research activities with the time he has freed up from the assistant director appointment. We look forward to working closely with Dr. Hooser, who is also head of the Toxicology Section of the Laboratory, in his new appointment.

On July 1, we also initiated a new segment in the life of ADDL, when we plugged in the new Aurora computer system in the Laboratory. We are struggling with it yet as this is written with everyone in the Lab getting acquainted with the system and the three people who adjust, instruct and rework areas of the system are making it better every day. Those involved most with the adjustments are Alan Bunning, Tim Kechkaylo and Jennifer Hewitt. Our thanks and accolades are out to these people, big time.

Have a good day. We look forward to assisting you with your diagnostic needs.

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FINAL DIAGNOSIS

Encephalitozoonosis

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

Signalment: 2 month old, female, New Zealand White rabbit

Clinical history: The owner has more than 150 rabbits. One to two rabbits have been dying every week for the past few months. Most have not had clinical signs. When clinical signs have been present, they included ataxia and weakness.

Gross necropsy findings: The capsular surfaces of the kidneys were pitted with multifocal pinpoint foci of fibrosis extending 1-3 mm into the cortex.

Histopathologic findings: Histopathology of the kidneys revealed multifocal lymphoplasmacytic interstitial nephritis with fibrosis. Moderate amounts of dense fibrous connective tissue and aggregates of mononuclear inflammatory cells infiltrated the interstitial spaces of multifocal wedge-shaped areas of the renal cortex. Histopathology of the cerebrum and cerebellum revealed a moderate, multifocal, granulomatous encephalomeningitis with associated *Encephalitozoon cuniculi*. Aggregates of mononuclear inflammatory cells, primarily macrophages intermixed with astrocytes and microglial cells, multifocally infiltrated necrotic (liquefactive) areas of the neuropil around the vasculature of the cerebrum and cerebellum. Astrocytes and microglial cells were moderately increased in numbers adjacent to the necrotic neuropil. A few cross sections of *Encephalitozoon* pseudocysts were multifocally distributed in the neuropil. The pseudocysts were irregularly shaped, approximately 15 to 30 microns in diameter, and contained several small spores. The spores were lightly basophilic, oval, and approximately 2.0 by 1.5 microns with a 1.0 micron purple-red, oval, internal structure in the center. Other changes included multifocal, lymphocytic perivascular cuffing and the presence of macrophages and lymphocytes multifocally lining the pia mater and filling the subarachnoid space.

Morphologic Diagnosis: (1) Multifocal, lymphoplasmacytic, interstitial nephritis with fibrosis and (2) Multifocal, granulomatous encephalomeningitis with associated *Encephalitozoon cuniculi*

Etiologic agent: *Encephalitozoon cuniculi*

Discussion: *Encephalitozoon cuniculi* are protozoa of the Microspora phylum. Microsporans are protozoa that produce unicellular spores, each containing a single, uninucleate sporoplasm. The spores usually have a complex extrusion apparatus that includes a hollow polar filament through which the sporoplasm enters a host cell. Microsporans are intracellular parasites of various organisms, including invertebrates, fish, and mammals.

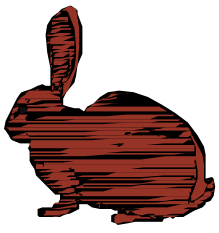
Encephalitozoon cuniculi are parasites of rabbits, mice, rats, squirrels, blue foxes, guinea pigs, dogs and humans. They have a direct life cycle that begins with the ingestion of a unicellular spore. The polar filament of the spore is extruded in the intestine, and anchors to the epithelium. The sporoplasm exits the spore via the polar filament into the cytoplasm of an enterocyte. The sporoplasm then multiples by binary fission and sporogony to form additional spores which are spread to various tissues of the body with a predilection for the brain and kidney. Other organs affected may include the heart, liver, spleen, lungs, and skeletal muscle. Spores are then transmitted through excretion in the urine with contamination of the environment, or through ingestion of an organism containing spores in its tissues. Urine and fecal transmission can be reduced if cages are kept clean and if sipper tubes or automatic waters and hopper feeders are used instead

of bowls or pans that are easily contaminated. Cages with wire bottoms elevated off the ground also help to reduce contamination.

In rabbits, gross lesions are usually not evident in the brain. The surface of the kidney may be multifocally pitted, representing cortical scarring. Histologically, a multifocal granulomatous encephalomyelitis and meningitis with lymphocytic perivascular cuffing is present. A multifocal granulomatous or lymphoplasmacytic interstitial nephritis may also be present, with spores in the tubular epithelium. Other lesions may include multifocal granulomatous inflammation and vasculitis with fibrinoid necrosis of small to medium arteries in the heart, liver, spleen, lungs, and skeletal muscle.

Encephalitozoonosis is usually chronic and insidious in rabbits, often without clinical signs. When present, clinical signs include weakness, ataxia and tremors. Encephalitozoonosis usually does not result in death unless a large parasite burden or underlying diseases are present. Clinical signs are worse in carnivores, especially in young puppies and foxes, and include stunted growth, renal failure, and neurological signs a few weeks after birth. Recent reports suggest *Encephalitozoon cuniculi* as the etiologic agent in a bovine abortion and an equine stillbirth. In addition to histopathology, serologic tests have been developed to detect *Encephalitozoon cuniculi.*

- by Matt Renninger, DVM
ADDL Graduate Student



ON THE ROAD

Drs. Leon Thacker, Charles Kanitz, Bill VanAlstine, Brad Njaa, and Marlon Rebelatto attended the North Central Conference of Veterinary Laboratory Diagnosticians at Michigan State University, June 2000.

Drs. Leon Thacker, Ching Ching Wu, and Tsang Long Lin attended the American Veterinary Medical Association and American Association of Avian Practitioners annual meeting in Salt Lake City, Utah, July, 2000.

Drs. Janice Lacey and Victoria Owiredu-Laast, ADDL Graduate Students, attended the Armed Forces Institute of Pathology Descriptive Veterinary Pathology course in Washington D.C., June, 2000

Dr. Ching Ching Wu served as technical committee Member at the National Poultry Improvement Plan Meeting in Colorado Springs, CO, June 2000

ADDL Graduate Students Drs. Janice Lacey, Victoria Owiredu-Laast, Karen Tucker-Gillum, Matt Renninger, Marlon Rebelatto, and Kaori Sakamoto presented papers at the Midwest Association of Veterinary Pathologists meeting in Bloomington In, August, 2000

Mary Woodruff, Virology Lab Supervisor, attended the Association of Veterinary Microbiologists meeting and workshops in Savannah GA, August 2000

Equine Exertional Rhabdomyolysis

A Thoroughbred racehorse acts colicky returning to his stall after a morning workout. An Arabian endurance horse quits at the 25th mile, trembling at the top of a hill. A Quarter Horse ranch horse is stymied by progressive weight loss and decreased performance. A Standardbred trotter lies sternal in his stall and is reluctant to rise. It's equivocal, but a Belgian draft mare seems to be showing a hind-limb lameness.

What might all these horses have in common?

It's possible that all five are suffering from Exertional Rhabdomyolysis, a clinical syndrome denoting muscular pathology, whose varied etiologies are only beginning to be determined. "Tying up", as the condition is commonly described, has been recognized by horsemen since at least the early part of the 20th century. Owners of draft teams, especially, noticed muscular distress in their horses following a full day of rest on full feed. Their workhorses came out stiff the "day after" (typically Monday), leading to the term "Monday Morning Disease". Curiously, not all draft horses were similarly affected. More curious still, not all affected horses were helped by a reduced diet during the rest day. The conclusion that "tying up" was associated with both heredity and husbandry governs research on the syndrome to this day.

Clinical Signs and Diagnosis

In general, exertional rhabdomyolysis elicits a spectrum of physical signs from mild stiffness and myalgia to recumbency and death. Diagnosis is based on clinical signs, with concurrent increases in serum creatinine kinase (CK), aspartate amino-transferase (AST), myoglobinuria in severe

cases, and distinctive lesions on muscle biopsy. Critical consideration must be given to ruling out other diagnoses. From the opening examples, it can be seen that rule-out diagnoses include colic, fractures, pleuritis, laminitis – anything that might cause a horse to act painful and reluctant to move.

Serum chemistry can help determine the prognosis as well as diagnosis. In a small study, one researcher noted that, while CK, AST, and lactate dehydrogenase (LDH) all increased immediately after onset of clinical signs, CK had the shortest half-life. AST and/or LDH, to CK, ratios corresponded with stage of disease. Ratios were low in acute stages, and high during recuperation.

Most diagnostic biopsies are taken from the middle gluteal and semimembranous muscles. These muscles are comprised largely of Type II (slow twitch or "non-oxidative") fibers; specimens are submitted on ice or frozen, not fixed. Histological signs of rhabdomyolysis (literally, "muscle cell loosening") include vacuolization and fragmentation of myocytes, and macrophage infiltration. Centrally located nuclei (indicating myocyte regeneration), fibrosis, and fatty infiltration all indicate chronic damage.

Pathogenesis

Of the many disparate causes for "tying up", certain ones are particular to certain breeds. The more heavily muscled, calmer breeds (e.g. Quarter Horses, warm bloods and draft breeds) are more likely to be affected by a defect in muscular metabolism called Polysaccharide Storage Myopathy (PSSM or EPSM). Thoroughbreds and other hot-blooded breeds, however, are thought to suffer a defect in intracellular calcium regulation. Other proposed etiologies tend to be suggested and unsubstantiated repeatedly, and include Vitamin E deficiency, electrolyte imbalance, viral infection, and, because of an increased incidence in mares, normonal influences.

The PSSM subset of rhabdomyolysis is named from an intramyocellular accumulation of periodic acid-Schiff-positive, abnormal mucopolysaccharide. Increased intra-cellular glycogen levels occur also, but are not specific to PSSM. Muscle glycogen levels may be more than doubled in PSSM positive horses compared to unaffected counterparts. It was originally thought that these horses suffered from a decreased ability to use stored glycogen; however, measurement of muscle glycogen and lactate during exercise tests have shown that the abnormality lies in increased synthesis, not decreased usage, of muscle glycogen. The role of the abnormal polysaccharide is unknown.

Affected thoroughbreds, and other breeds which "tie up" without histologic evidence of PSSM, have been shown to have abnormal regulation of myocyte contraction. *In vitro* studies compare muscle twitch responses to chemical stimuli such as caffeine and halothane. Muscle-contracture thresholds are lower in samples from affected horses, and the theory is that abnormal calcium regulation is to blame.

Genetic studies of affected horses have revealed different patterns of inheritance for both the PSSM and calcium related forms of exertional myopathy. PSSM horses have been shown to be descendents of one of three stallions, with an autosomal recessive inheritance pattern. The names of the three stallions have not been released. In thoroughbred racehorses, pedigree analysis revealed an autosomal dominant inheritance pattern, with variable expression. The trait seems to have first appeared in stallions in the early 20th century.

Treatment and Prevention

Horses acutely affected with rhabdomyoysis should be moved as little as possible. Mild cases may be walked bak to a stall, but movement will worsen the pathology in severe cases. In this regard, treatment should be directed towards reducing pain and anxiety in these horses to keep them as quiet as possible. Flunixin meglumine, phenylbutazone, acepromazine, and butorphanol have all been used with success.

Correcting hydration status is crucial, especially to prevent kidney damage in cases with myoglobinuria.

Dantrolene, a muscle relaxant which inhibits intracellular calcium release, and Phenytoin, an anti-convulsant, have been used for both treatment and prevention. No clinical trials have demonstrated efficacy, however, and dosing may be expensive and time consuming. One author suggested use only in horses known to be affected with abnormal intracellular calcium regulation.

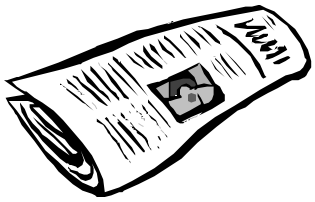
Limited studies have shown a decreased incidence of rhabdomyolysis following a switch to high-fat, low-carbohydrate diets, based on the theory that this will decrease muscle glycogen stores. Regular, daily exercise and turn-out are also suggested; their success in reducing episodes of rhabdomyolysis is anecdotal.

- by Katherine Ulman, Class 2000
- edited by Janice Lacey, DVM, ADDL Graduate Student

Purdue ADDL and Heeke ADDL
will be closed on the following dates

September 4, 2000	Labor Day
November 23,24 ,2000	Thanksgiving
December 22-26,2000	Christmas
January 1, 2001	New Year's Day

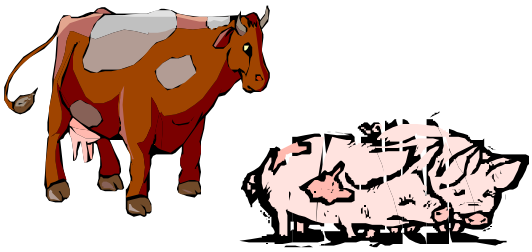
Please plan accordingly.



ADDL STAFF NEWS

The serology laboratory at ADDL has successfully completed USDA proficiency testing for Equine Infectious Anemia, Bluetongue and Bovine leucosis. These check tests are administered at regular intervals in order to maintain a laboratory's ability to conduct official tests. Serology technicians **Brenda Turner, Tammy Crowell** and **Jennifer Wells** are to be commended for their efforts.

Dr. Evan Janovitz, ADDL Pathologist, has served for six years as a member of the American College of Veterinary Pathologists (ACVP) examination committee. The ACVP is an organization of veterinarians who specialize in practicing, teaching and performing research in veterinary pathology. Diplomate status is attained by passing a certifying examination (CE), for which candidates qualify only after at least three years of supervised post-DVM study and training. The ACVP was established in 1949 making it the oldest of the current specialty colleges recognized by the AVMA. The Examination Committee consists of a group of ACVP diplomates selected to write, edit, administer, and grade the annual CE. In 1999, Dr. Janovitz was chairman of the Anatomic Veterinary Pathology Committee and will complete his service in 2000. The Examination Committee is active throughout the year, writing and editing questions during winter and spring, meeting in Colorado to assemble the CE in June, and administering the CE in Iowa in September. The veterinary pathology section itself is subdivided into clinical pathology and then (by the candidate's choice) three of the following five sections: dog and cat pathology, large animal pathology, laboratory animal pathology, wildlife and non-mammalian pathology, and toxicologic pathology.



UPDATE ON NEW TESTS FROM THE BACTERIOLOGY LABORATORIES AT ADDL

New antibiotic sensitivity panels for bovine and porcine isolates

Effective August 21, 2000 and in addition to our existing antimicrobial sensitivity break point (BK) panels (Table 1) for food animals (mainly bovine and porcine), the bacteriology laboratory will be offering a new panel (Table 2) which will provide minimum inhibitory concentrations (MIC). The cost for sensitivity tests remains the same if only one panel is tested for each isolate. Please indicate on your submission form if you wish to test your isolates against the MIC or BK panel. If not indicated, the BK panel will be used. As you will notice, the new panel has replaced tetracycline (TC) with chlortetracycline (CTC) or oxytetracycline (OTC). This change is based on recent data indicating that there are poor correlations between *in vitro* efficacy of TC and that of CTC or OTC. Since TC has not been effective and there are limited spaces on the plates, TC was removed from the panel. In addition, more sulfa drugs have been added to the new panel while amikacin, novobiocin, cephalothin and sarafloxicin were removed. The new panel will allow veterinarians to choose the most effective and economic antibiotics.

Bacteriology updates continued on page 6

Table 1 (Break Point)

Antibiotic	Concentration range (ug/ml)
Amikacin	16,32
Florfenicol	0,5,1
Apramycin	8,16
Novobiocin	4
Penicillin	0.03,0,12,2
Cephalothin	8,16
Tilmicosin	8,16
Clindamycin	0.5,2
Enrofloxacin	0.5,1
Erythromycin	0.5,4
Gentamicin	4,8
Oxacillin+2% Nacl	2,4
Ampicillin	0.25,2,4,8
Spectinomycin	8,16
Sulphachloropyridazine	20,40
Sarafloxacin	0.06,0.12
Tetracycline	4,8
Tiamulin	8,16
Trimethroprim/Sulphadiazine	0.5/9.5,2/38
Tylosin	5,10
Ceftiofur	1,2,4
Neomycin	8

Table 2 (MIC)

Antibiotic	Concentration range (ug/ml)
Ceftiofur	0.5,8
Erythromycin	0.25-4
Chlortetracycline	0.5-8
Florfenicol	0.25-8
Penicillin	0.12-8
Ampicillin	0.25-16
Apramycin	4-32
Sulphadimethoxine	32-256
Neomycin	4-32
Sulphachloropyridazine	32-256
Tylosin Tartrate	2.5-20
Sulphathiazole	32-256
Spectinomycin	8-64
Tilmicosin	4-32
Clindamycin	0.25-2
Tiamulin	4-32
Enrofloxacin	0.12-2
Gentamicin	1-8
Trimethroprim/Sulphamethoxazole	0.5/9.5,2/38
Oxytetracycline	0.25-8

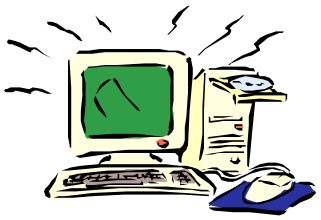
Johne's PCR

While we have passed the check test for Johne's culture by the National Veterinary Services Laboratory (NVSL) for the past three years with high marks, we still want to improve the turnaround time for diagnosing Johne's disease. A PCR test has been developed and validated. It will be applied to the bacterial culture tubes for fecal specimens at two to six weeks after incubation. Please note that we do not recommend eliminating the culture but, if you need a faster presumptive diagnosis, the PCR can help identify the positive shedders more quickly. Based on our experience, we can detect bacterial colonies at the 8 week culture tubes; however, we can detect positive results for Johne's disease by PCR from the same tube at 2-3 weeks after incubation before any colonies become visible. For low shedders, it generally takes 12-16 weeks for the bacterial colonies to be visible in the culture tubes; a swab taken from the same tube at 4-6 weeks after incubation would give positive PCR results for Johne's. If you need a quicker presumptive diagnosis, it would be beneficial to request PCR in addition to the routine culture request. There will be an additional charge of $15.00 for each PCR requested.

Information needed

We would like to take this opportunity to thank our users for their efforts in completing submission forms in detail and providing us with differentials whenever possible. This enables us to use the most appropriate reagents/media for bacterial isolation and identification. Please continue to provide with as much history, treatment and other relevant information as possible.

-by Ching Ching Wu, Head of Bacteriology



New Computer System-New Reports from ADDL

Why a new computer system?

Our old computer system was based on software that was developed before the evolution of Microsoft Windows and was not compatible with a Windows environment. The main computer that we used was dated and relatively slow. The old system did not allow for interfaces with an increasing number of electronic test devices that automatically transfer test results and other data via electronic links. Likewise, it did not have the potential for access to test results and reports by our users via the world- wide- web.

Our new system is based on modern programmable database software that is Microsoft Windows compatible and is able to utilize the power and capacity of more powerful computers. This system is a new product that has been purchased by several large veterinary diagnostic laboratories. Due to the specialized nature and limited number of veterinary diagnostic laboratories, each laboratory must customize the program to meet their specific needs. We are working with several other laboratories and with the software supplier to continue enhancement of this program and its capabilities. Our primary goal is to provide test results in concise user-friendly consolidated reports via current and emerging technologies such as automatic FAXing, automatic E-mail and/or 24-hour access via the world-wide-web. Look for additional enhancements in the future. Please call us with your concerns and suggestions.

Reading our new reports

Our reports are consolidated. This means that each time a report is printed it contains all of the results of all completed tests. We print and mail or FAX reports twice daily to ensure that results from tests completed in the morning will be sent by afternoon of the same day. The computer only prints a new report for each case if there are new test results since the previous printing. Although reports contain an increasing amount of repetition, new tests results for each laboratory always appear at the top and are dated. Therefore, by quickly scanning the top few lines for each laboratory and reading only results with the same date as the report date, one can easily find new test results. Reports are entitled "Preliminary Report" or "Final Report". All reports are "Preliminary" if there are any incomplete tests. When a report is "Final", all tests are complete. Of course, this means that the final report replaces all previous reports and would be appropriate for inclusion in a permanent client file.

For cases requiring gross or histopathology, the pathologist acts as an overall case coordinator and completes a "case summary" section for the report that will always be displayed first. The case summary is completed when histopathology and most other tests are completed and includes a final diagnosis(es) for the case as well as a comment that explains the rationale for the diagnosis or other opinions of the pathologist.

Most test results are reported as a concise single line in a standard format (see sample report). This format is as follows:

Sample (animal ID), test result by test type (date test completed).

Please note that the "animal ID" in parentheses behind the sample will be a number 1-6 if the number of animals represented in a case is less than or equal to 6. There is an animal ID "key" that appears at the top of the report immediately above the report title. This key makes clear which animal is which by listing additional unique identification for each animal numbered 1-6, such as color, ear tag number, tattoo number, etc. as indicated by the submitter. For cases that contain samples from large numbers (>6) of animals, such as milk samples from dairy cows or nasal swabs from pigs, the ear tag number or other unique animal ID that is submitted with each sample will be displayed on the report line in the parentheses instead of the numbers 1-6. This method for animal identification in our single-line test reporting format, although a bit confusing at first, was implemented because it seemed to provide the most accurate and concise method to report results from a variety of types of cases.

The bottom of each page of each report has a footer that contains the name of the submitting veterinarian and animal owner, the page number

and the ADDL case number. This allows easy identification of misplaced pages from reports.

Future Enhancements

We are working to improve this system, the format of our reports and the availability of results to our users.

In the near future, we intend to also include all tests that are pending on each report. These will be listed by laboratory at the first of each result block and will be clearly identified.

For example:

PENDING: Lung (1), virus isolation

We also intend to make it easier to identify new test results on each report by identifying only those tests with new results since the previous printing of the report with a unique initial symbol or by an obviously different format We are currently generating our antibiotic sensitivity reports as we did in the past by manually printing them from a separate machine. We hope soon to be able to transfer these sensitivity reports electronically into our new software so they will become part of our consolidated report. This is important for electronic viewing of reports via the world-wide-web.

As a longer-term goal, we and other laboratories that are implementing this software hope to develop an interface for the world-wide-web. This would allow users of our laboratory to access current and past reports 24 hours a day for their own clients. Of course, this feature must include security safeguard that ensure client confidentiality. It would also allow downloading of submission forms or electronic submission of submission data for animals being delivered by a third party.

Feedback

As you all probably noticed, we have had some growing pains as all of us learn to use the new program and input results of tests. Reports for the first few weeks often lacked uniformity. We are attempting to continuously improve our skills as well as the software. We need your feedback. Please let us know things that you like or dislike about our new reports. If you have suggestions for improvements, please share them with us.

-by Greg Stevenson, DVM, PhD
Professor of Veterinary Pathobiology,
Veterinary Pathologist, ADDL