UNIVERSITY of GUELPH LABORATORY SERVICES

Animal Health Laboratory

AHL Newsletter

Volume 6, Number 4, page 37 December, 2002 ISSN 1481-7179

In this issue:

AHL Christmas hours, 2002

The AHL will be **open with limited staffing 9AM - 5PM, December 27, 28, 30, 31.**

Phone extension changes

- Please note that effective January 1, 2003, **all UofG four-digit telephone extensions will have the number 5 added to the beginning.** The central switchboard will remain ext 0. Outside callers to the University will receive a greeting that directs them to first dial a 5 before dialing the campus extensions.

We are pleased to announce that **Dr. Sameh Youssef** joined the AHL in September, 2002, as an anatomic pathologist. Dr. Youssef graduated from the Alexandria Veterinary College (AVC) in 1990; his PhD (1997) in toxicologic pathology was a bilateral project with the Hanover Veterinary College. Dr. Youssef is currently completing his DVSc in anatomic pathology in the Department of Pathobiology, OVC.

Welcome Sameh!

Submission of carcasses

Tony van Dreumel *tvandreu@lsd.uoguelph.ca*

During the past few months, a number of severely decomposed carcasses have been submitted for necropsy. **Please note that the AHL may reject carcasses of animals that have been dead more than 24 hrs**, because such cases are usually unsuitable for diagnostic purposes, especially for histopathology and microbiology. Exceptions may be made under special circumstances such as Humane Society cases or other potential legal cases.

Some of the recent cases were submitted to AHL-Guelph on weekends with a request to delay the necropsy until the next regular work day to avoid the after-hours surcharge. The chance of making a diagnosis on such decomposed carcasses is often greatly compromised, which frustrates the pathologist, the referring veterinarian, and the owner. Remember that carcasses, especially larger ones and those with heavy fur or wool coats, continue to decompose even when refrigerated.

Cremation of animals

Linda McCaig *lmccaig@lsd.uoguelph.ca*

The Animal Health Laboratory is not a licensed crematorium and therefore does not return ashes to owners. **The owner must indicate 'private cremation' at the time the animal is delivered to the AHL for postmortem.** We will hold a body for private cremation for a limited time after postmortem. The body will only be released when the pathologist has collected all relevant samples and indicates that it may be released. A waiver releasing the AHL from liability must be signed at the time the remains are picked up.

It is the responsibility of the owner or the submitting veterinarian to make private cremation arrangements. Cremation is available through a variety of private services - e.g., see http://www.northhillswoodworking.com/cemetary.html

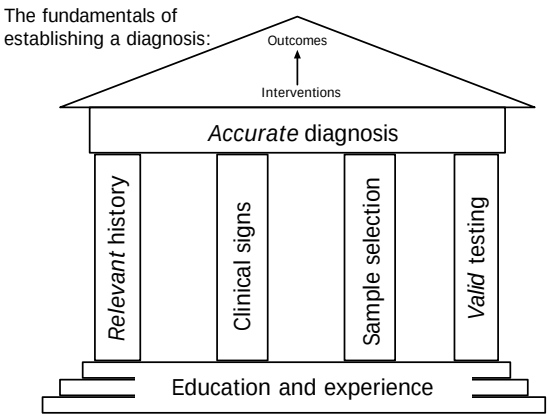
Agent, infection, disease - what does this test result really mean?

Grant Maxie *gmaxie@lsd.uoguelph.ca*

In an age of molecular diagnostic capabilities, laboratorians are able to detect increasingly small quantities of infectious agents. However, laboratory clients should note that detection of an agent by an exquisitely sensitive molecular technique does not necessarily mean that the agent is the cause of infection or of disease in an animal. Identification of a pathogen, e.g., *Mannheimia haemolytica* in the upper respiratory system of a steer, or circovirus 2 in a pig, does not establish this agent as the cause of disease. As well, other variables, such as sampling error or laboratory contamination, must be ruled out in order to gain better understanding of the significance of the agent identified.

Given these inherent difficulties, **how do we establish accurate diagnoses?** Based on our education and experience as veterinarians, we establish diagnoses, at least those involving laboratory findings, as depicted in the graphic to the right. According to Sackett et al. (1), there are 4 common diagnostic approaches:

1. *pattern recognition*, as used by seasoned clinicians;
2. the *multiple branching method of the delegate* (a junior person to whom you have delegated authority);
3. the *exhaustion method of the novice* (obtain a 'complete' history and do a 'complete' physical examination); and
4. the *hypothetico-deductive method*, which is the most widely used and involves forming a number of hypotheses based on experience and then ruling them in or out.



Diagnoses are typically supported by relevant history, clinical signs, and appropriate sampling and laboratory testing. There are a number of **prerequisites to obtaining useful test results**. These include:

- **selection of *appropriate* samples** - we give details of sample selection for a variety of conditions in the AHL User's Guide;
- ***proper* shipping and handling** - details are provided on our website and in the handout "Packaging and shipping lab submissions";
- **selection of *appropriate* tests** - if in doubt, call the lab!;
- **testing by *valid* methods**, as explored below; and

(continued on page 39)

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Editorial Assistant: **Ms. Helen Oliver**

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- ***valid* interpretation of results** - one test may be used to establish that 'infection' exists, but no single test should be used to define the existence of 'disease'.

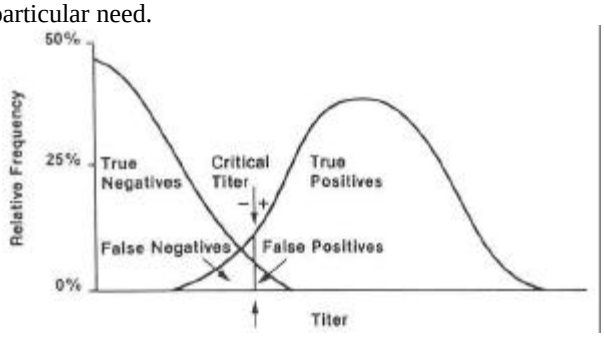
Validation of tests within the lab is a subject of great interest to laboratorians and of considerable significance to their clients. **Laboratorians and clients both want an assay that will accurately predict the negative or positive infection status of an animal or herd.**

Assay validation consists of a series of steps (2, 3):

1. *feasibility study* - e.g., a method is selected to detect antibody or antigen;
2. *the method is developed and standardized* - optimal conditions, analytical sensitivity and specificity, and repeatability are established, i.e., '**analytical validation**';
3. *the method performance is characterized* - we determine accuracy, precision, cut-off points, diagnostic sensitivity and diagnostic specificity (DSn, DSp, preferably with confidence limits), and compare the new method with a 'gold standard' - this is '**field validation**', which is expensive and difficult to perform given the large sample numbers needed, and hence is not often done;
4. *monitor performance of the method for validity* - estimate prevalence, calculate positive and negative predictive values (PPV, NPV); and
5. *maintain/extend validation criteria.*

Steps 1 and 2 are routine at the AHL; steps 3-5 are arduous.

With respect to serological tests, clients should be aware of how '**cut-off points**' ('critical titer' in the drawing below) are established, and how these can be altered to suit a particular need.



Based on testing of a reference population, the **cut-off point** is established by: visual inspection of the frequency distribution, by receiver operating characteristic (ROC) analysis, or by adding 2 or 3 SD to the mean of all negative values. **Note that the cut-off can be selected to suit the desired diagnostic sensitivity or specificity**, e.g., lowering the cut-off/critical titer in the diagram above will include more of the 'true positives' but will simultaneously increase the 'false positives'.

Ideally, we seek a test that will maximize the true positives and minimize the false negatives, i.e., has **high diagnostic sensitivity**, and we also want a test that will maximize the true negatives and minimize false positives, i.e., has **high diagnostic specificity**. Unfortunately, tests with 100% sensitivity and 100% specificity do not exist.

Ideally, we also want a test that has high positive predictive value (maximize true positives and minimize false positives) and high negative predictive value (maximize true negatives and minimize false negatives).

Although sensitivity and specificity are relatively stable for each test, **positive predictive value varies with prevalence.** For example, a test with 99% DSn and 99% DSp will generate only 1 false positive and 1 false negative per 100 tests from the target population (sounds excellent so far!!). However, in a population with a low prevalence of infection, e.g., 1 per 1000, the test will give 10 false positives per 1000 and 1 true positive. Hence the positive predictive value of this seemingly adequate test = (1 true positive) / (1 true positive + 10 false positives) = 9%. Interventions based on such a test must be carefully weighed, e.g., treating or euthanizing dogs or cats in an area of low heartworm prevalence based on a positive heartworm antigen ELISA alone would be ill-advised.

In surveillance programs, we can afford to use a test with high diagnostic sensitivity, or can lower the cut-off point, and hence generate false-positives, as long as we have available to us confirmatory tests with high diagnostic specificity. This is the case in our bovine spongiform encephalopathy program in which we use immunohistochemistry as a screening tool that will detect low levels of disease but will also generate false positives. We then refer these suspects to CFIA for confirmatory testing with Western blot or other tests with greater diagnostic specificity.

Can we succeed in disease diagnosis and control efforts with less than perfect tests? Yes, we need only look at the success of brucellosis and tuberculosis eradication in Canada. **It must be remembered that no test is perfect or is free-standing**; testing should be incorporated as an integral part of a management protocol. For example, Johne's disease testing, although imperfect, (ELISA, ~98% DSp, but < 50% DSn; fecal culture, >99% DSp, but 70-90% DSn) can be used effectively in an overall management program to eliminate Johne's disease from carefully managed herds.

The good news is that **testing options are improving** as laboratorians increasingly become aware of the need for, and the complexity of, test validation. There is also increased awareness among clinicians of the need for critical assessment of test results. As well, inter-laboratory standardization and collaboration are improving through the efforts of organizations such as the Canadian Animal Health Laboratorians Network and the American Association of Veterinary Laboratory Diagnosticians. *AHL*

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AHL Lab Reports

CATTLE

Botulism in cattle

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Tony van Dreumel, Marie Archambault, Beverly McEwen, Tom Wheal, Peggy Jo Darden

Botulism is a serious disease of humans and animals, caused by one of the most potent toxins known. Of the eight known types of *Clostridium botulinum* toxins (A, B, C_1, C_2, D, E, F and G) (1), **types B, C, and D are most commonly associated with outbreaks in cattle** (2). A predominant toxin is usually produced, but some strains can produce more than one. The toxins block acetylcholine release from cholinergic nerve endings. The disease is usually characterized by flaccid paralysis. Ingestion of preformed toxin in feed, usually silage, baylage or contaminated litter, is the most common route of intoxication (2). Cattle can also acquire botulism by ingestion of small animal carcasses, such as rodents or birds, which can be present in feed.

Botulism can be prevented by proper storage of feed.

Since 1998, 25 cattle have been submitted to the AHL with a tentative clinical diagnosis of botulism. The animals came from 11 different farms. The clinical diagnosis was based on sudden onset of weakness leading to paralysis, lateral recumbency, and often death within 24 – 48 hours. Paralysis of the tongue was reported in five animals. A total of 170 cows had died and 29 more were down in the affected herds at the time the animals were submitted to the laboratory. The majority of the affected animals were adult dairy cows.

In an attempt to confirm a diagnosis of botulism, a mouse inoculation test (MIT) was done using sera, and rumen or intestinal contents from 10 affected animals; 5 tested positive. Cross-protection with specific *C. botulinum* antitoxins showed that 3 animals had *C. botulinum* type D and 2 had type C. The rest of the submitted animals likely had botulism based on typical clinical signs, absence of lesions to explain the paralysis, and in some cases additional negative tests for other toxins such as ionophores and organophosphate insecticides.

A recent serious outbreak of botulism in a large dairy herd follows:

- A free-stall dairy herd of 250 milking cows had sudden onset of drop in production - some cows were found dead and others developed progressive paralysis, including reduced tone of the tongue - 40 cows died or were euthanized during a period of three weeks.
- Four cows were submitted for necropsy, and none had lesions to explain the paralysis. Rumen content from 2 cows was inoculated into mice. One of these tested positive for *C. botulinum* type D and negative for types B and C.
- Shipment of milk was suspended immediately after a tentative clinical diagnosis of botulism was made in the herd. Milk from 6 affected cows and 2 bulk tank samples were tested using MIT, and all mice survived. Milk shipment was resumed only after no new cases of botulism were diagnosed.
- The herd was vaccinated with *C. botulinum* toxoid C and D 6 days after the index case and again 3 weeks later. No new cases were diagnosed after the second vaccination. **The source of the toxin in this outbreak was not determined.**
- The animals were fed a mixture of corn silage, baylage and brewer's grain. Initially brewer's grain was suspected as the source of the toxin and it was immediately removed. However, more cases of botulism occurred for several days after the brewer's grain had been removed. Two goats fed the brewer's grain on the premises remained healthy. Feeding of baylage was discontinued approximately three weeks after the onset of the outbreak. The baylage was stored in long plastic tubes, and it has previously been identified as a major risk factor for botulism in cattle (2).

This outbreak illustrates the serious losses of animals and production that may be associated with this disease. **Botulism should be suspected in any herd in which a number of animals have sudden onset of weakness leading to paralysis and recumbency. Loss of lingual tone is highly suggestive of botulism but unfortunately it is inconsistent.**

A standard protocol during an investigation of a suspected outbreak of botulism in a herd should include submission of affected animals and suspect feed to a laboratory. MIT is not a perfect test, but it is currently the most sensitive test available for confirmation of botulism. Samples from acutely affected animals are more likely to contain toxins.

The provincial veterinarian should be notified as soon as botulism is suspected in a herd because of potential public health concerns. **Botulism can be prevented by proper storage of feed.** *AHL*

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Trypanosome infection in an Ontario cow

Brent Hoff, Lynn Olds, Debbie Hood, James Dykeman *bhoff@lsd.uoguelph.ca*

A 5-year-old red Holstein cow, in a herd of 40, was fresh several months and 44 days pregnant. She had a fever, marked pallor of mucous membranes, and large petechiae on vulvar and nasal mucosae. The laboratory data indicated marked anemia (hematocrit 0.10), thrombocytopenia, and neutropenia. **There were many large trypanosomes present in blood smears as well as in wet mounts** (0.45 X 10^9/L) (see Figure 1 below). Based on size and shape, the organisms were identified as *Trypanosoma theileri*.

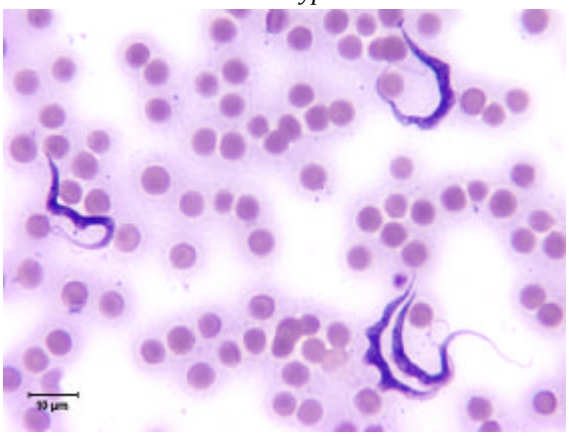

Figure 1. *Trypanosoma theileri* in a bovine blood smear.

***Trypanosoma theileri* is a cosmopolitan parasite that has been described from every country where cattle are found.** *T. theileri* is one of the largest mammalian trypanosomes, measuring 60-100 µm long. Although *T. theileri* has been known for more than 90 years, there is still some controversy regarding its effect on the bovine host. Most reports claim that the parasite is completely harmless, but some veterinarians believe that it is potentially pathogenic, and that its pathogenicity can be activated by intercurrent disease.

This cow had a selective deficiency of IgG_2, with only 1.04 g/L (ref. 5.0-13.5 g/L). It is interesting to note that this condition has been reported in red Danish cows that showed increased susceptibility to mastitis and various other infectious conditions, such as bronchopneumonia, peritonitis and abomasoenteritis. The cow in our case had severe enteritis.

A survey of 15 dairy herds in Ontario in 1970, by Dr. R. J. Julian, found 54% of herds to be positive for *T. theileri* parasitemia. Sampling of several other animals in the herd with this infected cow found no parasitemia in any other animals. With supportive therapy, the affected cow had a slow but complete recovery, with no parasitemia. *AHL*

Progressive ataxia in a Charolais steer

Sameh Youssef, Tony van Dreumel, Sharon Puente *syoussef@lsd.uoguelph.ca*

Multiple tissues from an 18-month-old Charolais steer were sent to AHL-Guelph for histologic examination. The steer had been thin, recumbent and unable to rise. Significant lesions were present in sections from the brain, wherein numerous multifocal basophilic plaques (Figure 1) were present in the white matter of a cortical section, and to a lesser extent in the obex. These plaques were homogeneous to granular and were surrounded by mild gliosis.

Similar lesions have previously been reported in Charolais cattle, and are considered pathognomonic of progressive ataxia of Charolais cattle (PACC) (1, 2). Other non-specific cerebral lesions included mild lymphocytic cuffs, astrocytosis, and axonal swelling with spheroid formation. PACC was first reported in the UK and has now been found worldwide. This fairly novel neurologic disorder is presumed to have an inherited basis. Ultrastructural studies have revealed that the plaques represent oligodendroglial dysplasia (1).

Differential diagnoses of the bovine neurologic diseases that are most common in Ontario include polioencephalomalacia, rabies, and lead toxicosis. PACC is characterized by late clinical onset (usually at 1-2 years) and a progressive gradual course. *AHL*

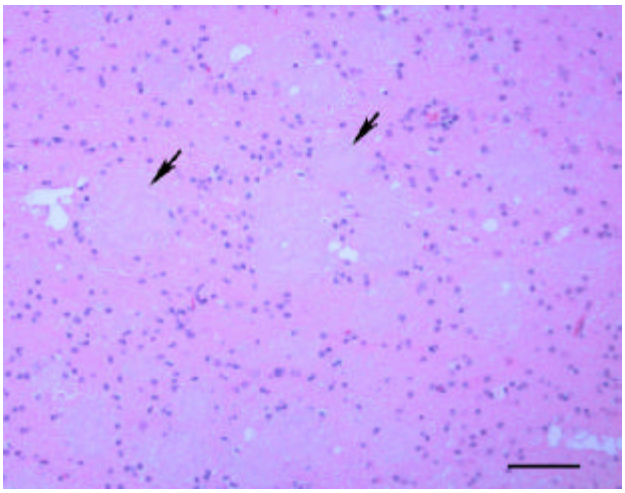

Figure 1. Multiple basophilic plaques (arrows) in the white matter of the cerebral cortex. Bar = 150 µm.

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Highlights from AHL-Kemptville – diagnoses in cattle

Jan Shapiro, Beverly McEwen *jshapiro@kemptvillec.uoguelph.ca*

Over 500 post-mortem cases were submitted to AHL-Kemptville in fiscal 2001/2002. Most of these were dairy cattle (55%), followed by beef cattle (14%), chickens (12%), and horses (10%). The remainder were cervids, small ruminants, swine, turkeys, and companion animals.

Abortion, gastrointestinal, and respiratory diseases were most common in food animals. There were 115 diagnoses made from the 90 cases of **bovine enteric disease** from 84 herds. Although **BVDV continues to be the most frequently identified enteric pathogen in these cases**, diagnoses of BVD-associated gastrointestinal disease have decreased since 2000/2001 from 24% to 14.8% of diagnoses. This is also lower than the overall 19% BVDV pathology diagnoses for the entire AHL.

In 15 of 28 neonatal dairy and beef calves submitted that developed diarrhea before 14 d of age, **enteritis** was associated with multiple pathogens, including enterotoxigenic *E. coli* (ETEC), rotavirus, coronavirus, adenovirus, BVDV, and cryptosporidia. When **ETEC** was involved, either alone or as one of multiple pathogens, herd neonatal calf crop mortality was 45-100%. **Adenovirus infection** was also diagnosed in 2 young calves associated with severe erosive enterocolitis - the herds practiced annual vaccination with killed multivalent vaccines including BVDV. Adenovirus infection is usually subclinical; clinical disease may be linked to immunosuppression.

In 11 of 13 dairy and beef cattle that developed diarrhea between 2 wk and 6 mo of age, **BVD** was diagnosed, either by isolation of cytopathic or non-cytopathic strains of virus (4 cases) or by histopathology (7 cases). BVDV was the only pathogen implicated in 7 of the 11 cases, with co-infection with *Salmonella* Typhimurium, coccidia, *E. coli* and cryptosporidia in 4 cases. In 6 dairy and beef cattle over 6 mo of age, BVD was diagnosed in 3, with isolation of cytopathic or non-cytopathic BVDV. Enteritis due to *Salmonella* Kentucky, BCV or *Clostridium perfringens* was diagnosed in the other 3 cases.

Sarcosporidiosis (Dalmeny disease) was diagnosed in 2 dairy cattle from a group of 10 sick heifers and dry cows that had been fed moldy bagged corn silage beginning 1 mo prior to death. The primary clinical complaint in the group was weight loss, depression and poor appetite. Immunosuppression due to BVDV was suspected in both cattle necropsied.

There were 67 diagnoses made in 63 cases of **bovine abortion** from 56 herds. ***Neospora caninum*** continues to be the single pathogen most frequently associated with bovine abortion, accounting for 15% of diagnoses, which is slightly higher than the 12.5% for the entire AHL. Other abortifacient pathogens sporadically identified at AHL-Kv included *Listeria monocytogenes*, BVDV, *Leptospira* spp., and *Ureaplasma diversum.*

There were 66 diagnoses made in 55 cases of **bovine respiratory disease** from 49 herds. *Mannheimia haemolytica* was identified more frequently in AHL-Kv pathology cases (14%) than overall (9.2%), whereas diagnoses of bovine respiratory syncytial virus infection was lower – 5% compared to the entire AHL pathology diagnostic rate of 9.9%. *Mycoplasma bovis*-associated enzootic pneumonia was 8% for both AHL-Kv and overall.

The frequencies of these diagnoses from AHL-Kv are not prevalence estimates of disease in eastern Ontario due to submission biases to the laboratory. However, they do provide some indication of pathogens and disease processes in the region. A sudden increase in particular diagnoses is a warning signal that further investigation is warranted to determine if these changes are artifactual or represent a true trend in disease. *AHL*

Sudden death in 'entrapped' cattle submitted to AHL-Kemptville

Jan Shapiro, Philip Watson, Tom McLean, Alan Darch *jshapiro@kemptvillec.uoguelph.ca*

Between October 1, 2000 and September 30, 2002, there were 15 beef cattle and 38 dairy cattle of various ages submitted to the AHL-Kemptville with a history of sudden death. Only cattle that were found in locations or in positions that suggested entrapment, such as being cast, stuck or hanged are included in this report. In all cases, owners indicated that the cattle were not observed to be ill prior to being found dead. In most cases, owners were submitting cattle as a requirement of their livestock insurance policy.

Of the 53 animals submitted, 48 (90%) had no significant primary gross or histological lesions, but several had findings, such as bloat, aspiration pneumonia, trauma or intestinal accident, that were considered to be secondary to being entrapped or cast. However, 3 of 15 (20%) beef cattle and 2 of 38 (5%) dairy cattle had primary lesions that were considered to be the cause of sudden death and that existed before the entrapment incident.

Unexpectedly, there were more lactating cows entrapped than cows that were heavy in calf and with presumably less agility. Also, slightly larger numbers of cattle were entrapped outside (30) compared to in free-stall, stanchion or tie-stall housing (23).

Some of the **risk factors** associated with entrapment in free-stalls or tie-stalls are slippery footing; type of bedding material; stall length; height of the head rail; distance between the head rail and the wall; location, angles and height of stall dividers; height of trainers; animal density; health of the feet; and the presence of metabolic or other disease. *AHL*

Mycoplasma bovis mastitis

Tony van Dreumel, Lois Parker *tvandreu@lsd.uoguelph.ca*

Mastitis associated with *Mycoplasma bovis* has been reported in many countries. The disease causes considerable loss of production in affected herds (1). Australian workers have recently shown a clear association between *Mycoplasma bovis* udder infection and elevated somatic cell counts (SCC) (2).

The clinical signs of mastitis due to *M. bovis* are non-specific. There is usually a sudden drop in milk production of the affected quarter(s). The appearance of the milk varies considerably from being watery to purulent (Fig. 1). The affected quarters are firm on palpation. There is usually no response to treatment. Agalactia is often the end result, and affected cows are culled for that reason. A few cows may have concurrent pneumonia and/or arthritis.

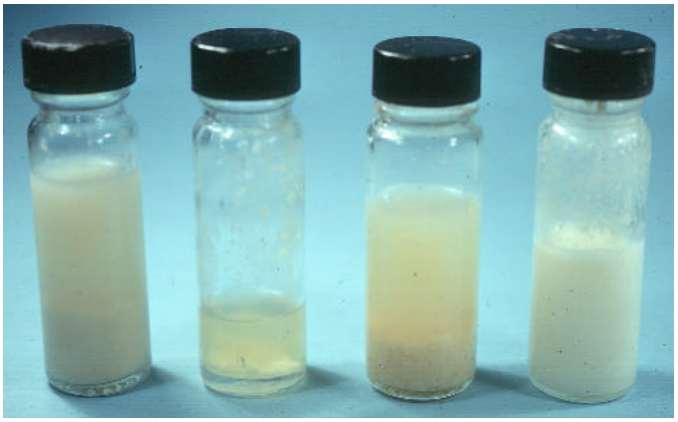

Figure 1. Milk samples from cow with *Mycoplasma bovis* mastitis. Note watery fluid on top of flocculent sediment.

Routine cultures of milk samples will not detect *Mycoplasma bovis*. Special media and laboratory procedures are required to isolate and identify the organism.

During the year 2001-2002, the mycoplasmology laboratory at the AHL received 138 milk samples and 18% of these yielded *M. bovis*. Of these milk samples, 87 came from individual cows (17% positive), 25 were bulk tank samples (16% positive), and the status of eight samples was unknown (12.5% positive).

In 2000/2001, 59 milk samples were submitted and none was positive. Whether the increase in isolation reflects increased incidence in the field is not known. Some workers have indicated that mycoplasma mastitis is more prevalent in areas with intensive milk production and high concentration of dairy cows, i.e., large herds (1).

Mycoplasma mastitis should be considered in any herds in which:

a. routine milk cultures fail to yield bacterial pathogens,
b. response to antibiotic treatment is poor, or
c. SCC's are persistently high and other causes of the elevated counts have been eliminated.

Milk samples submitted for mycoplasma culture should be clearly marked to indicate whether they are from individual cows or bulk tanks. Requests to culture for mycoplasma must be clearly made on the submission form. Samples should be shipped on ice and arrive at the laboratory within 24 hours of collection. Frozen samples are acceptable if shipment takes more than 24 hours. Duplicate milk samples should be submitted if culture for both mycoplasma and bacterial pathogens is desired. *AHL*

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Somatic cell counts

Peter Lusis, Marie Archambault *plusis@lsd.uoguelph.ca*

Somatic cell counts (SCC) can be very useful in interpreting the significance of milk bacteriology culture results.

Environmental pathogens (coliforms, environmental streptococci, yeasts, and others) isolated from milk samples may be contaminants if SCC's are low (less than 200,000-300,000). In these cases, treatment is unnecessary and results in discarding normal milk due to antimicrobial withdrawal times.

Especially in chronic cases, many pathogens (coagulase-negative staphylococci, mycoplasma, mycobacteria, anaerobes, and others) may not be isolated from infected quarters using single-sample routine procedures and should be suspected if SCC's are high and bacteriology is negative. **Quarter (not composite) samples from cows with high SCC's should be cultured 2 to 3 times at 3 to 4 day intervals - these samples can be kept frozen until submitted for culture.**

Streptococcus agalactiae in milk is significant regardless of SCC.

In summary, important pathogens (e.g., coagulase-positive staphylococci) can be missed, or unnecessary treatments administered (e.g., environmental bacterial contaminants), if SCC's are not done for routine herd mastitis testing. **Please remember that frozen samples are not suitable for SCC testing.** *AHL*

AVIAN

West Nile virus in captive bird species in Ontario

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West Nile virus (WNV) has had a dramatic impact on numerous wild bird species in Ontario during 2002. Surveillance of corvids (crows, blue jays) demonstrated the presence of WNV in Ontario during 2001. **In the summer and fall of 2002, the incidence of WNV infections in non-corvid wild bird species, particularly raptors, has increased dramatically.** Dr. Douglas Campbell of the Canadian Cooperative Wildlife Health Center in Guelph has recently reported the occurrence of WNV in non-corvid species including the following: red tail hawk, great horned owl, Cooper's hawk, sharp-shinned hawk, American robin, osprey, ring bill gull, great black-backed gull, and Canada goose.

> West Nile virus should be added to the list of rule-outs in cases with neurological signs or sudden death in free-living and captive bird species.

West Nile virus infection and disease is occurring not only in free-living birds but also in birds that are raised in captivity. West Nile virus has been demonstrated by three different methods at the AHL:

- an antigen capture ELISA (VecTest, West Nile/Saint Louis Encephalitis Antigen Panel Assay, Medical Analysis Systems Inc., Camarillo, CA, USA) on tissues and fluids,
- an immunohistochemical (IHC) stain utilizing a polyclonal antibody to demonstrate the West Nile flavivirus antigen in formalin-fixed tissues, and
- inadvertently by isolation in embryonated chicken eggs.

The first two methods have been undergoing validation at the AHL while the third, virus isolation for WNV, is not conducted at the AHL because the necessary level 3 facilities are not available. Immunohistochemical identification of WN viral antigen in tissues has been particularly useful in avian cases where the antigen load is usually greater than in mammalian tissues. IHC not only allows direct visualization of the tissues and cells that are infected but it also provides a subjective assessment of the viral antigen load - monocytes/phagocytes are the cells most commonly involved.

A captive **American kestrel** that was brought to the Veterinary Teaching Hospital at the Ontario Veterinary College by a falconer was shown to have WNV by the inadvertent isolation of the virus and by IHC. A **red-shouldered hawk** that had been raised in captivity was also positive for WNV by the VecTest and IHC staining.

Two **Atlantic Brant geese** were submitted to the AHL because of a sudden onset of nervous signs and mortalities in a group of 80 captive bred and raised geese. Some of the birds demonstrated torticollis and loss of balance for 1 to 1.5 days prior to death. A few birds did recover partially after an extended period of supportive care. There were no significant gross necropsy findings, and swab samples of brain, liver and spleen of both birds were negative with the VecTest. Histological examination demonstrated single-cell hepatocyte necrosis with accumulations of foamy macrophages containing pale yellow pigment and debris in the liver, spleen and around a few blood vessels in the brain. West Nile virus was unintentionally isolated on second passage of brain tissue in chicken embryos. The lesions in the embryos and a positive VecTest on allantoic fluid were indicative of WNV infection. There was strong IHC staining in heart and kidney consistent with abundant viral antigen.

West Nile virus resulted in sudden death in a group of 5-month-old **canaries**. Approximately 20 of 40 juveniles died while adult breeders in close proximity were unaffected. The young canaries were lethargic for 18 to 24 hours prior to death. The birds were housed outdoors in a screened 'partly mosquito-proof ' cage. At necropsy, spleens were 2 to 3 times normal size and livers were pale and mottled by red foci. Histologic examination demonstrated foamy debris-containing macrophages in the liver, spleen, and bone marrow, and around a few blood vessels in the brain. Lymphoid necrosis was present in the intestine and in splenic lymphoid sheaths. Samples of liver and spleen were positive for WNV on the VecTest, and IHC was positive in lung, air sac, liver, kidney, heart, intestine and brain. Interestingly, feather/quill mites were present on and within the feathers of several birds. It has been suggested that blood- and tissue-fluid-feeding ectoparasites might serve as vectors for WNV in closely confined groups of birds.

In another case, 3 of 9 3-year-old **parakeets** that were housed in a garage died suddenly. No abnormalities were observed prior to death. Mild to moderate splenic enlargement was the only consistent finding on gross necropsy in the 3 birds that were examined. Splenic necrosis was seen in one of the birds and light accumulations of foamy macrophages and lymphocytes were present in liver and spleen sections. The VecTest was positive on pooled samples of brain, liver and spleen. Positive IHC staining was demonstrated with moderate to heavy staining in sections of heart, intestine, liver and light staining in the brain. *AHL*

Investigation of infectious bursal disease virus and bursal lymphoid depletion in Ontario broiler chickens - results to date

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Bursal lymphoid depletion continues to be a common finding in broiler chickens from 3 weeks to market age. To investigate the possible role that infectious bursal disease virus (IBDV) may play in bursal lymphoid depletion, we have conducted more in-depth testing. During the past year, in collaboration with biologics companies, poultry practitioners, hatcheries and feed companies, we have evaluated weekly samples from flocks experiencing production problems and/or increased mortalities. Samples were taken on a weekly basis from birds that were 2 to 5 weeks of age.

> Variant viruses, including Delaware E-like viruses, have been present in Ontario chickens for several years.

By utilizing bursal histopathology, serology (both a standard IBDV ELISA kit and an extended range IBDVxr kit that appears to be more suitable for the detection of antibodies to variant viruses), and molecular techniques such as RT-PCR/RFLP analysis and gene sequence analysis, we have evaluated these samples for the presence of field IBD viruses. **It is evident from these studies so far that bursal lymphoid depletion in broiler chickens is frequently associated with an IBD virus challenge.** Bursal damage usually occurs between 2 to 4 weeks of age when maternal antibody titers are low.

Of the IBD viruses analyzed to date, the majority are classified as "variant'' viruses. We now know that variant viruses, including Delaware E-like viruses, are present in Ontario chickens and, by testing archived bursal samples, we have shown that these viruses have been present in Ontario for several years. Analysis of Ontario IBDV strains is continuing. Further evaluation of the IBDV status in poor- and well-performing flocks is being planned. *AHL*

SWINE

Leptospira pomona outbreak

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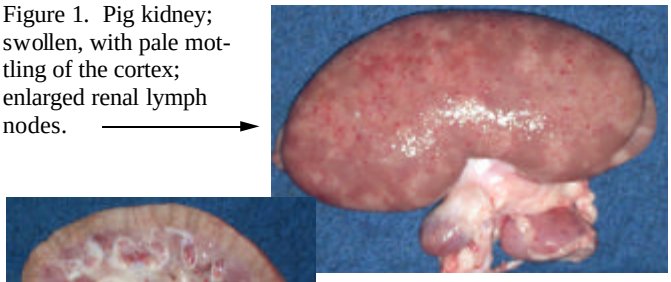
From January 1 to August 28, 2002, a total of 68 carcasses from a 900 sow farrow-to-finish herd had been condemned at slaughter, due to "glomerulonephritis". Diagnostic services were requested in late August, when the weekly condemnation numbers rose from 1, to 2, to 7, and finally to 17, in the approximately 100 hogs that were shipped each week. Representative kidneys were submitted to the AHL from pigs slaughtered the following week. On gross examination, kidneys varied from slightly shrunken to 1.5 times normal size, and invariably had pale mottling that extended through the cortex to involve the medulla (see Figures below). Microscopic examination revealed tubulointerstitial nephritis, with lymphoid nodules apparent in affected kidneys. Renal lymph nodes were 3-10 times normal size, often having a cystic appearance.

The production unit consisted of two 450-sow operations, with farrowing and nursery rooms located on each site, and these operating on an all-in, all-out basis. Piglets, after leaving the nursery units, were sent to 1 of 4 continuous-flow, bioshelter-style grower barns, located off-site. At approximately 80 kg body weight, pigs were moved to one of 4 continuous-flow finisher barns, again off-site, with 2 barns in 1 location and the other 2 at separate sites. The affected pigs were thought to originate from the site with the 2 barns, one of which was a conventional naturally ventilated barn and the other a bioshelter.

Figure 1. Pig kidney; swollen, with pale mottling of the cortex; enlarged renal lymph nodes. →



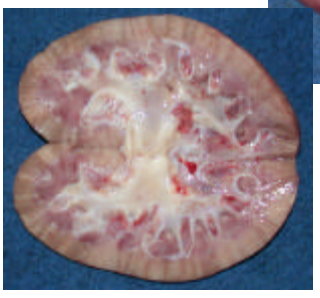


Figure 2. Cut surface of pig kidney, with pale radial streaking of the cortex/medulla. ←

(continued on page 46)

Leptospira pomona outbreak (continued)

Waste cereal products, such as Fruit Loops, were fed to all pigs. These were collected and stored at a central site, and then transported to each barn. Water was considered to be a problem both in regards to quality and quantity - wastewater from a rice processing company was purchased, stored in large tanks, and used in all of the barns.

A *Mycoplasma hyopneumoniae*-related respiratory problem had been diagnosed several months earlier. The owner however, was not able to detect any "sick" pigs prior to them being marketed.

Serum samples were submitted from pigs in both barns at the suspect site, from pigs at another finisher operation, and from 12- and 18-week-old grower pigs.

Kidneys from slaughtered pigs were positive for circovirus type 2 using polymerase chain reaction (PCR) testing, and negative for parvovirus by the fluorescent antibody technique. One of two pools of urine, aspirated from submitted kidneys, was **positive for *Leptospira*, using PCR** (validated for dog urine only). **Kidneys were positive for *Leptospira* spp. using immunofluorescent stains**, and spirochetal organisms were identified in tubular cells with **Warthin-Starry staining** techniques.

Serum samples from a total of 30 pigs from both barns on the suspect site were **positive for *Leptospira pomona* using the microscopic agglutination test**, with titers ranging from 1:640 to 1:20480. A serological response was not identified in sera from 34 pigs from the other finisher barn and from grower pigs. All serum samples were negative for *Leptospira grippotyphosa*. Interestingly, pigs from all groups had titers against *Leptospira bratislava*, with titers higher in pigs from the suspect farm. These titers were considered to be due to cross-reactions to the *L. pomona* infection.

Tetracycline was administered in the water, and the condition responded well. Three weeks later, only 1 pig was identified at slaughter with renal lesions.

The original source of the infection was not identified. Pigs, however, are maintenance hosts for *Leptospira pomona* and, once infected, can shed large numbers of organisms in the urine, in spite of having markedly elevated antibody levels.

The unique set of circumstances in this case that included the moving of pigs into the continuous-flow finisher barn at 80 kg no doubt contributed to the longevity of the outbreak, and also to the fact that clinically affected pigs were not observed prior to marketing.

Leptospirosis in pigs, although not common, does occur, and when it does, can be an economically significant disease. *AHL*

HORSES

Ataxia, fatal pulmonary edema, and lympholysis in adult horses

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Two recent equine necropsy submissions to the AHL have had a history of acute-onset of dyspnea and ataxia, progressing to death in less than 12 hours. Histologic lesions common to these cases were severe and widespread lymphoid necrosis involving lymph nodes, spleen, and thymus, and diffuse pulmonary edema with lymphocytic perivasculitis. Intranuclear inclusion bodies consistent with herpesvirus were present in a few scattered lymphocytes surrounding areas of necrosis. Fluorescent antibody testing for EHV-1/4 was positive in lung from one horse.

The clinical findings and histologic lesions suggest that **these cases may represent an unusual manifestation of equine herpesvirus (EHV)-1 infection**. Additional tests to confirm this diagnosis are currently underway on these animals and on two additional horses with similar clinical and necropsy findings. EHV-1 infection in adult horses is commonly associated with respiratory disease and encephalomyelopathy; generalized disease with prominent lymphoid necrosis, as seen in the recent AHL cases, is more often associated with fetal and neonatal herpesviral infections.

Practitioners should be aware of the possibility of atypical clinical presentations of EHV-1 infections in Ontario horses. The diagnostic outcomes of these cases will be presented in the next issue of the AHL Newsletter. *AHL*

Reference

Del Piero F, et al. Fatal nonneurological EHV-1 infection in a yearling filly. Vet Pathol 2000; 37: 672-676.

West Nile virus infection in horses in Ontario, 2002

Josepha DeLay, Murray Hazlett, Susy Carman, Scott Weese, Paul Innes *jdelay@lsd.uoguelph.ca*

Ontario veterinarians experienced a dramatic increase in cases of acute neurologic disease in horses from late August to October of this year. **Of the equine neurologic cases submitted to the AHL during this period, over 90% (44 horses) have been confirmed as West Nile virus (WNV)-infected horses.** WNV infection has been identified in horses stabled in the regions of Hamilton-Wentworth, York, Niagara, Haldimand-Norfolk, Toronto, Halton, Waterloo, Wellington, Northwestern, Ottawa, Perth, Oxford, Brant, Lambton, Chatham-Kent, and Windsor-Essex; **the majority of cases occurred in horses in southwestern Ontario**. Typical clinical signs observed included an acute onset of pyrexia, muscle fasciculations, ataxia, and lethargy, often rapidly progressing to recumbency within 24 hours. Of the 37 confirmed WNV-infected horses for which clinical outcome is known, 22 horses died or were euthanized (case fatality rate = 59%).

> Serology remains the most conclusive test for the confirmation of WNV infection in horses with clinical signs consistent with this disease

Ante-mortem diagnosis of WNV infection in horses with these clinical signs involved demonstration of IgM antibody to WNV by antibody capture ELISA (confirming a primary immune response), in conjunction with an elevated titer of WN viral-neutralizing antibody (IgG) in serum (Cornell University Diagnostic Laboratory, Ithaca, NY). Alternatively, the diagnosis can be confirmed by demonstration of a 4-fold or greater rise in IgG neutralizing antibody to WNV, using paired sera collected 2-3 weeks apart. To date, 27 confirmed cases of equine WNV infection have been diagnosed through our laboratory by serology alone. There were 10 additional horses with clinical signs suggestive of WNV infection and high levels of IgG antibody, although IgM results were unavailable or inconclusive.

WNV infection was identified in 17 horses submitted for necropsy; 15 of these necropsies were performed at the AHL/OVC, and 2 animals were necropsied by referring veterinarians, with tissues submitted for histologic evaluation and additional testing. Test results from 3 additional necropsy cases were suspicious for WNV infection. All horses examined had clinical signs consistent with published descriptions of WNV infection. Rabies was the primary differential diagnosis in each case and was excluded by testing at the Canadian Food Inspection Agency (CFIA). No gross lesions were evident in the majority of cases; two horses had malacia or hemorrhage in thoracic and lumbar spinal cord. Histologic lesions were concentrated in brainstem and spinal cord, with rare cerebral cortical involvement, and consisted of mild to severe multifocal non-suppurative encephalomyelitis with perivasculitis and gliosis (Figure 1). Specific diagnostic tests for WNV infection on each horse included various combinations of ante-mortem serology, immunohistochemistry (IHC) for WNV antigen in formalin-fixed tissue sections with histologic lesions (performed at the AHL), and polymerase chain reaction (PCR) testing for WNV genomic material in frozen sections from brainstem (Veterinary Services Branch, Manitoba Department of Agriculture, Winnipeg, MB). For 7 cases, all 3 tests were positive; a diagnosis of WNV infection was reached in the additional cases by one or more positive results from PCR, IHC, and/or serologic tests.

Distribution of WN viral antigen in infected horses is limited to the CNS and is extremely sparse, in contrast to the multi-organ distribution of abundant virus seen in birds. For this reason, IHC or PCR can produce false-negative results if the sample chosen for testing does not contain sufficient virus for detection purposes.

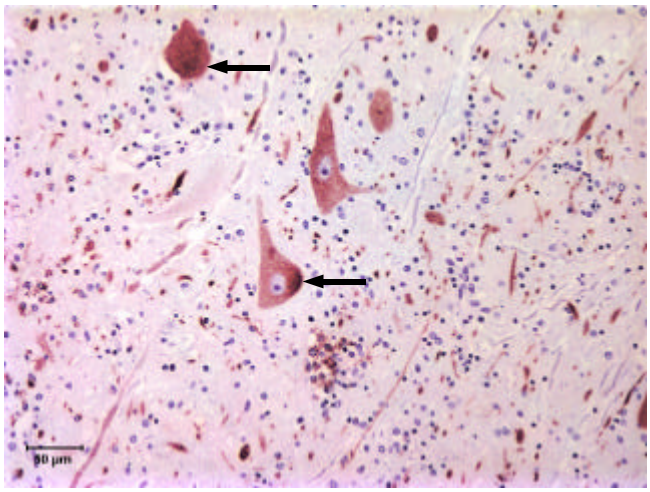

Figure 1. West Nile virus antigen in neuronal cell bodies (arrows) and glial cells in medulla; gliosis. Immunohistochemistry.

To increase the chance of virus detection, multiple sections of formalin-fixed brainstem and spinal cord should be submitted for immunohistochemistry. Although histologic examination, IHC, and PCR are very useful adjunct tests, when combined, for the diagnosis of WNV infection, **serology remains the most conclusive test for the confirmation of WNV infection in horses with clinical signs consistent with this disease.** *AHL*

Reference

Cantile C, Del Piero F, Di Guarido G, Arispici M. Pathologic and immunohistochemical findings in naturally occurring West Nile virus infection in horses. Vet Pathol 2001; 38: 414-421.

COMPANION ANIMALS

Ethylene glycol toxicosis

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Ethylene glycol (antifreeze) toxicosis is the most commonly diagnosed cause of poisoning of dogs and cats at the AHL. In a recent case, several dogs, cats and wild animals were suspected of being maliciously poisoned - two dogs were submitted for necropsy. Both had severe oxalate nephrosis. Their stomachs were empty because of vomiting, but high levels of ethylene glycol (2100 ppm) were demonstrated in gastric mucus. In another case, suspect feed was identified because it had not frozen in spite of very low (winter) environmental temperatures.

Animals find ethylene glycol palatable and 2-5 mL/kg is fatal to most dogs and cats. Death usually occurs within 2-3 days as a result of direct neurotoxicity, metabolic acidosis, hypocalcemia, and renal failure (1). Blood and urine may contain ethylene glycol and/or oxalates, but **oxalate crystals may not be present in the urine of some poisoned animals, and the presence of oxalate crystals in urine is not in itself diagnostic of oxalate toxicosis because they may be present from dietary sources in the urine of 'normal' animals.** Oxalates are formed during the metabolism of ethylene glycol and are not present in ethylene glycol or in the suspect source of poisoning.

Treatment of dogs with adsorbents, emetics and fomepizole (4-methylpyrazole, Antizol-Vet, Orphan Medical Inc., Minnesota) within 8 hours of ingestion is often effective. Fomepizole does not seem to be effective in cats (2). For cats, treatment with adsorbents, emetics and intravenous ethyl alcohol within 3 hours of ingestion is often effective.

Histologic examination of kidneys is diagnostic in these cases, but, if malicious poisoning is suspected, the suspected source and stomach and intestinal contents should also be submitted for toxicologic confirmation for possible legal proceedings (OSPCA/Humane Society, OPP). *AHL*

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1. Osweiler GD. Toxicology. Lippincott Williams & Wilkins. 1996: 319.
2. Plumb DC. Veterinary Drug Handbook. 4th edition, Iowa State University Press. 2002: 391-392.

Questions, comments, suggestions for the editor??

- Questions about services provided by the AHL?
- Suggestions for topics to be covered in future editions of the Newsletter?

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Season's Greetings from the staff of the Animal Health Laboratory