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Animal Health Centre

Ministry of Agriculture, Food and Fisheries

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From the Director

R.J. Lewis:

Readers may be aware of the outbreak of exotic Newcastle Disease (END) now underway in southern California. Newcastle disease exists in several forms and we occasionally diagnose a mild form of this disease in BC. The severe, or velogenic, form (END) is differentiated from the less severe strains by high death losses, rapid spread, and specific laboratory virus testing. END was first identified in backyard game fowl in southern California last fall and rapid spread has led to infection in seventeen commercial poultry operations. These operations have been or are presently undergoing depopulation; the disease has now been recognized in game birds in Nevada and Arizona. To date, over three million birds have been humanely destroyed in an attempt to eradicate the disease. END is considered a "foreign" animal disease so the US Department of Agriculture, in cooperation with the California Department of Food and Agriculture, is involved in the investigation and identification of affected birds. Quarantines, market loss, and the large loss of birds result in heavy economic consequences for both producers and the state.

Similar to our rapid reaction to the foot-and-mouth disease crisis in the UK, the ministry has also responded to the request for assistance with the eradication of END. Dr. Victoria Bowes, avian health veterinarian for the Animal Health Branch, has just returned from two weeks assisting in these eradication efforts and gaining hands-on experience in dealing with a foreign animal disease outbreak. She is sharing this information with staff directly and will also be speaking within the next week at two poultry commodity group meetings. Her valuable experience will be of great assistance to our industry should we ever experience a similar disease outbreak in BC. A future issue of *Diagnostic Diary* will include an overview by Dr. Bowes of her experiences and what we can learn from that outbreak.

With the longer days and clear signs of Spring occurring, there will also be a return of mosquitoes within the next several weeks to many areas of British Columbia. It is anticipated that this year we will make the first identification of West Nile Virus (WNV), carried by mosquitoes, in this province. The virus spread extremely fast almost all the way across

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TOLL FREE NUMBER AND WEB SITE:

Please note that the Animal Health Centre has a toll free number: 1-800-661-9903. Keep this in mind, if calling long distance. This Newsletter, and other information from the AHC, may also be found on the Internet at our web site : http://www.agf.gov.bc.ca./croplive/anhlth/ahc

North America last year and was identified in Washington State late in the year. With funding from Health Canada, the BC Centre for Disease Control and the Animal Health Branch are preparing surveillance efforts to ensure early detection of WNV. Health units across the province are being prepared to field calls from people reporting dead birds to them. Crows and other corvids (such as Stellar's jays, blue jays, and ravens) are extremely susceptible to the virus and face high mortality if bitten by an infected mosquito. As it has been demonstrated that WNV is usually discovered in birds several weeks in advance of a human infection, birds may be used as a form of early warning. Birds from health units will be couriered to the Animal Health Centre for evaluation for WNV. Staff is being hired under contract to perform a rapid test to identify the presence of the virus; if this rapid test is positive, molecular diagnostics (polymerase chain reaction) will be used for immediate confirmation. The BC CDC will be notified and that information will be communicated to the affected health unit to ensure people are aware that WNV is present in their area. Should you encounter dead crows or other members of the corvid species, please contact the health unit in your area for advice.

Dr O'Toole's West Nile Virus seminar at the AHC:

Dr. Donal O'Toole, Director of the Wyoming State Diagnostic Laboratory, spoke at the Animal Health Centre on January 23 on West Nile Virus (WNV) and other equine health concerns. The response to distributed notices of his WNV talk was excellent; the lecture theatre within the AHC was packed with equine and large animal practitioners, representatives from the Canadian Food Inspection Agency (CFIA), laboratory staff of the AHC, animal health technicians , and Dr Helen Schwantje of the Ministry of Water, Land, and Air Protection.

Dr O'Toole recounted the rapid spread of WNV across the American mid-west in 2002, and the ensuing kerfuffle; the State of Wyoming experienced more than 100 clinical equine cases. O'Toole presented an informative history of the disease in both Africa and North America. His Wyoming experience with WNV was graphically demonstrated via a video of clinically affected horses. Dr O'Toole recommended that horses be vaccinated according to the manufacturer's instructions prior to the start of mosquito season. Discussions between Dr O'Toole and attending veterinarians on reports of alleged WNV vaccine reactions generated much interest from everyone.

In addition to his seminar on West Nile Virus, O'Toole also discussed equine rabies and the tendency for this disease in some instances to become manifest clinically as a spinal problem in horses, rather than the more typical behavioral signs seen in other animals. On other topics, his discussions on equine surgical dermatopathology evoked numerous questions and comments from the attending practitioners.

In addition to his meeting with practitioners, Dr O'Toole presented several seminars on a variety of topics to AHC pathologists during his visit. These included his own experiences in the study of malignant catarrheal fever (MCF) in bison and other species, vaccine reactions in domestic livestock, recent reports on the etiology of equine protozoal myelitis, as well as discussions on congenital neurological diseases in beef cattle including Maple Syrup Urine Disease. A video tape on bioterrorism was presented to AHC staff for later review.

A gifted speaker, Dr O'Toole's informative, interesting, and colourful presentations were enthusiastically received by everyone.

Report on the safety of WNV vaccine administration to horses:

In response to a written request by Dr Ann Britton, pathologist at the AHC, Dr Steven Jacobs of Wyeth Animal Health (Canada) stated (in an e-mail note) that of the hundreds of thousands of doses which have been administered to horses in Canada since conditional release of the vaccine, only two reports of hives and three reports of injection site reactions have been received by the company. There were also five reports of horses clinically affected with signs resembling WNV, that had been vaccinated during the outbreak. Any additional questions regarding the safe use of this particular vaccine may be referred to: Dr. Steven Jacobs, BSc, DVM, Professional Services Veterinarian,Wyeth Animal Health, 400 Michener Road Guelph, Ontario N1K 1E4 .Tele. 1-800-267-1777 ext 25, or fax: 519-837-1876.

Animal Health Monitoring Lab (AHML) news:

L Curley, Manager

a. West Nile Virus (WNV) - Capture ELISA for equine IgM:

West Nile Virus is receiving much attention in British Columbia, with most authorities predicting positives in the province by this summer. The AHML is now ready to test for equine IgM to West Nile Virus. Whereas incubation can be five to fifteen days, IgM antibody is present by eight to twelve days post-exposure, and can persist from two weeks to two months. Horses are a dead end host and there is low viremia. In the USA there has been a 33% fatality rate in horses that become clinically ill. A confirmed case consists of compatible clinical signs plus one or more of the following: isolation of the virus, IgM in serum or CSF, or IHC positive.

If WNV is suspected following clinical examination of an ill horse, a blood sample should be drawn as early as possible. If the horse recovers, a second sample can be taken two or three weeks later.

There has been concern that vaccination will preclude the use of this ELISA as a diagnostic tool, but recent correspondence on the AAVLD (American Assoc. of Vet Lab Diagnosticians) list indicates that the vaccine does not provoke IgM response, only IgG. U.S. labs are continuing to use the IgM capture ELISA on clinically suspect horses, vaccinated or not, with confidence.

b. Caprine Arthritis-Encephalitis (CAE) and Maedi-Visna (MV or OPP) cELISA:

At the request of the CFIA Retrovirology Centre of Expertise, the AHML tested a panel of 40 caprine sera and 40 ovine sera, using the CAE/OPP competitive ELISA. This test had 100% correlation with the CFIA in-house ELISA test (Simard), with no CV (coefficient of variation) greater than 9%. As a result of this validation, the CFIA is now referring veterinarians for diagnostic testing of CAE and MV to our laboratory.

c. *Coxiella burnetii* (Q Fever) blood test:

We offer a blood-serum antibody test for *C. burnetii*, using IFA (immunofluorescence) slides coated with both Phase I and Phase II antigens. Using slides specifically blocked for bovine vs ovine/caprine sera, and the proper conjugates, this test can be performed for all three species. Use of these slides was validated by sending split bovine serum samples to the University of Maryland laboratory, and correlating results.

d. Malignant Catarrhal Fever (MCF) cELISA*:*

A new test available at the AHML is the competitive ELISA for Malignant Catarrhal Fever (MCF). This is a disease of bison and cattle, but sheep carry this herpesvirus without showing clinical signs. The test of choice for clinical disease is PCR; serology (antibody detection) is best used for surveying the level of latent infection in a population. Specificity of the cELISA exceeds 98%, and it has been found to detect 80 to 90% of diseased cattle and 60 to 70% of diseased bison. Because bison have a higher fatality rate and a more rapid course, there is a lower percentage of seropositivity in these animals at death. Competitive ELISAs can be used for any species, thus sheep can be tested as well.

For more information on these tests or other serology available at the AHML, please call 604 556-3135.

EQUINE ROUNDUP :

Ann Britton

This is the first edition of EQUINE ROUNDUP, a summary of equine cases submitted to the Animal Health Centre. Since starting my position as veterinary pathologist at the AHC three years ago, I have periodically reviewed equine submissions for my own interest and this column is a natural evolution of that process.

In this inaugural column, submissions from mid-October to the end of December 2002 have been tabulated by category of diagnosis in Table 1. No surprises here - out of a total of 26 submissions, gastrointestinal problems and abortions accounted for over half of the cases.

Gastrointestinal problems broke down into two distinct diagnoses – necrotizing colitis/ enterocolitis and intestinal accidents.

Acute severe inflammation of the intestinal tract (necrotizing colitis/enterocolitis) was diagnosed in 4 horses (two 5 month-old foals from the same farm, and one 17 year-old and a 7 year-old horse from unrelated farms). In only one case was a specific pathogen associated with the lesions – one of the 5 month-old foals had a significant bacterial overgrowth of *Clostridium spp*. observed on the mucosal surface, and a presumptive diagnosis of clostridial colitis was made.

In cases of necrotizing colitis and enterocolitis, clostridial disease should always be on the list of differentials. As *Clostridium spp*. overgrowth is a common post mortem change observed in the intestine, arriving at a definitive diagnosis in these cases can be challenging. Microscopic changes compatible with clostridial disease - severe damage to the lining of the intestine or colon with little inflammatory response and overgrowth of large numbers of clostridial bacteria directly on the surface - is usually sufficient for a presumptive diagnosis when no other intestinal pathogen is isolated.

The risk factors for acute equine diarrhea are not well understood. In acute clostridial diarrhea leading to death in other species, there is usually a history of abrupt change in diet such as increased consumption of carbohydrates, which favour clostridial overgrowth. In the horse it would appear that changes in management which cause unaccustomed stress, are more important. Recent transportation, antibiotic administration, surgery, and mild respiratory illness are stressors often linked to the development of acute equine diarrhea.

The following observed intestinal accidents had all been preceded by severe colic:

– gastric (stomach) impaction and dilation in a one year-old
– epiploic foramen entrapment of small intestine with gastric rupture in a 20-year old
– intestinal displacement and necrosis associated with adhesions in a 21 year-old
– entrapment of the colon in a mesenteric rent diagnosed in a 20-year old

Of six abortion cases, a diagnosis was made in two – *Streptococcus equi zooepidemicus* was isolated in significant numbers from one case, and umbilical torsion was diagnosed in the other.

Three CNS cases were submitted:

– a two year-old wobbler in which no lesions were detected
– a 22 year-old with non-suppurative encephalitis from which no pathogens were isolated
– a 7 year-old with mild non-suppurative leptomeningitis (inflammation of membranes overlying the brain tissue) from which no pathogens were isolated.

Two bacterial cases were examined on necropsy:

– a yearling with bacterial septicemia linked to an *Actinobacillus suis*-like organism
– a 13 year-old with a ruptured pituitary abscess

Two horses were euthanized due to musculo-skeletal problems. One animal had a carpal fracture and the other had osteochondrosis of the shoulder with severe secondary arthritic changes. Osteochondrosis is a degenerative disease of bone tissue involving the growth ossification centres.

Three tumors were diagnosed including metastatic hemangiosarcoma in a 28 year-old. Two biopsies were submitted for diagnosis – spindle cell tumour and melanoma.

The remaining cases comprised a horse which had been seized from its owners following death from starvation, and a five year-old with dermal habronemiasis or "summer sores" (inflammation of the skin with larvae from the nematodal parasites *Habronema spp*).

Highlight Case - ruptured pituitary abscess in a thoroughbred mare:

Arguably, the most interesting case involved a 13 year-old Thoroughbred mare with a ruptured pituitary abscess. This mare was presented to the clinician with depression and an inability to lower her neck. The animal had a nasal discharge and a body temperature of 104F. She was treated with antibiotics as well as anti-inflammatories and Banamine to no avail. The mare became restless then recumbent and was finally euthanized within 24 hours of initial examination.

At necropsy, major findings were restricted to the cranial vault. The meninges were markedly congested; upon hemisection of the brain, purulent material oozed from the ventricular cavities. Thick purulent material could also be expressed from the infundibulum of the pituitary, and the pituitary per se was adhered to the ventro-lateral membranous dura via a 1 cm-diameter abscess. There were no lesions in the guttural pouch, inner ear, teeth, sinuses, or oral cavity. The left nasal cavity contained purulent material immediately proximal to the cribriform plate.

Histologically, the brain lesions reflected a necrotizing and suppurative imflammatory process compatible with a bacterial etiology; the pituitary had been nearly destroyed by inflammation. Large numbers of gram positive streptococcal organisms were demonstrated microscopically within the inflammation foci. Because no primary inflammatory focus could be found, it was concluded that the infection may have arisen due to localization of circulating *Streptococcus spp*. in the pituitary. Alternatively, it was suggested that the infection may have ascended via the Eustachian

tube as remnants of the tube were observed in the peri-pituitary inflammation histologically. Bacterial inflammation within the tube may then have spread to the adjacent cavernous sinus and involved the pituitary secondarily.

I hope that this first edition of EQUINE ROUNDUP has been of interest, and I look forward to reviewing future equine cases in the AHC newsletter.

Short Cuts from the Post Mortem Room:

J Coates

Fowl cholera ***(Pasteurella multocida)*** was diagnosed in a flock of 200 male broiler breeders (33 weeks), in which prominent thick caseous deposits of fibrin enveloped the lung pleural membranes on gross necropsy. Lung tissue in these birds was variably congested and meaty in texture suggestive of pneumonia, and fibrin clots were observed within the peritoneal cavity. Pleural exudate yielded a heavy growth of *Pasteurella multocida* on culture. Viral studies were negative. Bacterial culture of pleural exudate, tracheas, and lung was negative on bacterial culture for *Ornithobacterium rhinotracheale (ORT),* although tissues were positive for this organism via the PCR technique.

Passage of dark blood-colored urine (**post-parturient hemoglobinuria in cattle**) was diagnosed in a mature dairy cow that had aborted a late-term, stillborn fetus. Several other potential causes of bloody or wine-coloured urine, including the bacterial disease leptospirosis, were ruled out in this case. There were few significant findings in the tissues of the cow's stillborn calf, other than degenerative changes observed in the placenta.

This syndrome is frequently (but not always) linked to chronic phosphorous deficiency in mature pregnant cattle. The syndrome is most commonly seen in the immediate post-parturient period. This animal had been exposed to very inclement weather prior to the acute onset of illness. In any one animal, the acute syndrome - as seen in this case - might follow exposure to excessive exercise or to drinking very cold water (Radostits et al: *Veterinary Medicine*, 9th edition). In grazing cattle, there is an inverse relationship between the incidence of the disease and soil fertility. Post-parturient hemoglobinuria syndrome tends to be sporadic, although it may affect a number of animals within a fairly short period of time. As a precaution, a nutritional review of this herd including mineral supplementation was recommended. This animal was not copper or selenium deficient. Liver copper levels were slightly elevated at 132.0 ppm wet weight, but not within the toxic range for cattle (Puls RE: *Mineral Levels in Animal Health,* 1994). Fetal liver copper was adequate at 61.0 ppm.

Listerial meningoencephalitis (inflammation of the brain and brain covering) was diagnosed by the veterinary practitioner in a **mature ewe** that had demonstrated clinical signs of circling, drooling, and facial edema; a mature ram in the same flock was showing similar clinical signs. A light growth of ***Listeria monocytogenes*** was cultured from the ewe's brain. Microscopically, brain tissue revealed a meningoencephalitis primarily within the brain stem, with random abscesses in the midbrain. Necropsy revealed no other significant lesions in this animal. The veterinary practitioner was immediately informed as to the potential for additional cases within this flock. Prior to the ewe's illness the weather had been cool, wet, and changeable.

The bacterial organism ***Actinobacillus suis*** was cultured in heavy growth from the lungs of a three month-old feeder pig that had died overnight; the animal was from a

large group of feeder pigs recently experiencing acute mortality. The lungs were diffusely firm and meaty on cut section, with the most severe changes in the anterior lung lobes. Hemorrhage into lung tissue was noted on cut section, and lung lobes were adhered together. The other most likely bacterial agent to produce a similar type of lesion is *Actinobacillus pleuropneumonia*e.

Severe **bronchopneumonia** was observed in the lung tissue of three week-old goat kids. ***Pasteurella multocida*** was cultured from the lung tissue in heavy growth. These three small kids also had very mild heart and skeletal muscle lesions indicative of a myopathy, that was linked to vitamin E-selenium deficiency.

A highly malignant diffuse peritoneal tumor resembling a **mesothelioma**, was observed grossly and microscopically in a 71 kg, 12 year-old **Vietnamese pot-bellied pig**. The peritoneal membranes of the abdomen had a thickened, cobblestoned appearance. The spleen, liver, and stomach were enveloped by this thick fibrous tissue. Microscopically, the densely cellular tumor consisted of interweaving bundles or whorls of mesenchymal-type cells that demonstrated a high mitotic index.

Coxiella abortion (***Coxiella burnetii***) was diagnosed in an aborted seven-month gestation Jersey fetus. The abortion was the first recently observed by the owner. Microscopically, the principal finding was severe inflammation of the placenta with occasional hemorrhages. Placental tissue was positive for *C burnetii* via the PCR test.

The presence of C *burnetii* in cattle is evidently more widespread than the abortion rate from this organism would indicate. The organism is potentially infectious to humans, known as Q (Query) fever.

In this case, the farm family had been drinking fresh, non-pasteurized milk. Because *C burnetii* may be excreted in the milk of lactating cows, a risk to human health exists. The simplest way to avoid this potential health hazard with cattle (or goat/sheep) dairy products is to drink only pasteurized milk and milk products. Other infectious organisms that are capable of remaining viable in non-pasteurized milk include bovine tuberculosis, leptospirosis, listeriosis, toxoplasmosis, and various bacterial coliforms and staphylococci.

Neospora protozoal abortion (***N caninum***) was diagnosed in a mid-gestational Holstein fetus from the BC Lower Mainland. Principal diagnostic findings included characteristic brain changes, marked liver inflammation, and a patchy, severe, infection of the heart muscle.

Microscopic alterations in the heart **muscle of an aborted six month-gestation Holstein fetus** were linked to a suspected fetal (in utero) nutritional myopathy, associated with vitamin E-selenium deficiency. There were no other definitive lesions seen on this aborted specimen, with no evidence of an infectious etiology. Placenta was not available for examination.

Fatal parvoviral enteritis was diagnosed in a 9 week-old Pomeranian pup. The history indicated acute gastroenteritis with vomiting, followed rapidly by death within 24 hours despite efforts to save the animal. Microscopic examination of the small intestine indicated severe inflammation of the intestine, with widespread necrosis of the glandular crypts. The virus was identified by the PCR technique, and via the fluoresecent antibody (FA) procedure. The animal also had extensive, widespread lesions of **lung mineralization,** linked to **over-dosage with vitamin supplements, particularly vitamin D.** Except for as few foci of kidney mineralization, other organs did not appear involved. This small puppy only weighed a few ounces; the potential for vitamin toxicity was readily apparent.

Nasal carcinoma was diagnosed in a mature, **free-ranging male black bear** (***Ursus americanus***) that had been shot by a conservation officer. The animal had been displaying nervous signs clinically, including a tendency to circle. The carcass was received completely frozen. On necropsy a yellowish-white, spongy tumor mass was observed within the posterior aspect of the nasal turbinate bones of the skull. The mass had infiltrated the skull and distorted the sphenoid bone immediately below the floor of the cranial cavity, in the area of the pituitary gland. Entry or infiltration of the tumor mass into the cranial cavity per se, or within brain tissue, was not detected. The frontal sinus of the skull also demonstrated a more localized but structurally similar tumor mass. There were no specific microscopic lesions detected within the brain tissue. Decomposition was prominent. No spread of the tumor to other body organs was found. In addition to the tumour, most of the upper right canine tooth was missing, having been sheared off at its base at a much earlier time. Similarly, the right eye had been severely damaged in an earlier incident, leaving only a shriveled fibrous remnant of the eye within the base of the empty ocular cavity.

Literature reports of tumors in bears are very few, and most are evidently malignant, as in this case. Liver and bile duct tumors have been unexpectedly frequent in captive bears (*Diseases of Wildlife in Wyoming,* 2nd ed. 1982; Wyoming Game and Fish Department, State of Wyoming, Special Publications Section).

BC Cheese Manufacture, 2002 - a public health concern:

J Waddington

In the past year, two separate incidents of food-borne illness caused by the bacterial organism *Listeria monocytogenes* in cheese have involved both the Food Safety and Quality Branch of the MAFF and the Animal Health Centre (AHC). In both cases the potential link to the farm environment was quite direct. Our role was to investigate this possible linkage, and to coordinate our findings with the emerging bacteriological picture gathered by the BC Centre for Disease Control (BC CDC)

In February 2002, an outbreak of human infection with *L. monocytogenes* was linked to a small cheese manufacturer on Vancouver Island. A total of 47 human cases were eventually identified, with 10 of these being invasive infections. The most serious human case resulted in meningitis and abortion. In September 2002, a second outbreak was again linked to a (different) small cheese manufacturer on Vancouver Island. No invasive cases were noted, but a total of 82 human cases of febrile gastroenteritis were linked to one particular cheese product.

Bacteriological studies identified *Listeria monocytogenes serotype 4b* as the causative organism in both cases, with strain differences identified by pulse field gel electrophoresis (PFGE) and ribotyping. In the first outbreak, the bacterium was identified in very high concentrations in a variety of cheeses made over a period of time at the identified cheese plant. In the second case, the organism could only be found in one particular cheese product, from one specific batch date.

In both outbreaks of human disease, livestock were resident on the premise where the cheese was being manufactured. In the first outbreak however, the resident animals did not supply the milk for the cheese making. Instead, goats' milk was purchased from a single producer, while pooled cows' milk was bought from a wholesaler. In the second case, milk used in the cheese-making process was obtained from a small herd of milking cows that were housed and milked on site.

L. moncytogenes (for convenience, referred to as *Listeria*) is relatively common among farm animals and an attempt was made to determine if the agricultural aspects of

these enterprises were involved in harboring and transmitting the infecting organism to the cheese plant.

Briefly, *L. monocytogenes* is a gram-positive bacterium that is environmentally ubiquitous. Common reservoirs are soil, water, domestic and wild birds and animals, as well as humans, where approximately 10% of the population are thought to be fecal carriers of the organism. In the farm environment, contamination of silage with *Listeria*-laden soil may occur. In addition, improper silage fermentation may result in a pH greater than 4.5, which actually promotes bacterial multiplication. *L. monocytogenes* can grow at temperatures ranging from 4° C (refrigeration temperature) to 45° C, and at pH 4.5 to 9.6.

Listeria commonly infects ruminants where it may cause disease of the nervous system, and occasionally abortions. Clinical signs frequently observed are head tilt, circling, difficulty in swallowing, and eventual recumbency. In people, listerial infection commonly causes gastroenteritis, but can occasionally take a more invasive, septicemic route that results in meningitis, as well as abortion in pregnant women.

Transmission of *Listeria* bacteria to humans most commonly occurs through food. Sources are ready-to-eat deli meats, some vegetables, shellfish, and dairy products. Although not a large proportion of the food-born illness cases ($\sim$ 0.02% of all food born illness), listerial infection results in $\sim$ 3.8% of hospitalizations, and 27.6% of the deaths occurring from food born illness. The estimated annual cost related to human listeriosis in Canada is estimated at 11 to 13 million dollars.

Because of the ubiquitous nature of *Listeria* in the environment, contamination of raw (non-pasteurized) milk is relatively common. Surveys in North America estimate the contamination rate at between 1.5-11.0% of raw milk samples. It's noteworthy that conventional pasteurization techniques are very effective in destroying this bacterium in dairy products. Although raw milk cheeses are made and consumed in BC, both of the plants involved in these outbreaks had been pasteurizing all milk prior to use for cheese manufacture.

The source of contamination was identified in the first outbreak. In the manufacture of certain varieties of cheese, it is standard practice to use a ripening solution of bacterial culture. This solution is made from commercially available lyophilized powders, which are re-constituted with water prior to use. In this case, *Listeria* contamination of the ripening solution had occurred, which was exacerbated by the fact that the solution was repeatedly 'topped-up', rather than a new batch being re-constituted within a sterilized container. As a consequence, large numbers of *Listeria* had been sprayed onto the cheese as a part of the ageing process.

In the second case, no point source of contamination was identified, although the same serotype of *L monocytogenes* had been cultured from the farm environment, and from the onsite human-waste septic tank system. Due to the use of water in the washing process of the culture-positive cheese, it has been postulated that water was the source of contamination. It is suggested that the well water used in the cheese plant may have become intermittently contaminated by farmyard run-off, secondary to a structural problem with the well position and casing.

In both of these outbreaks, the combined cooperative and investigative efforts of the CFIA, (Canadian Food Inspection Agency), the BC Centre for Disease Control, public health authorities, and the MAFF's Food Safety and Quality Branch rapidly identified the problem. In addition to minimizing the public health impact of listerial outbreaks, useful information has followed for the individual cheese plants in question and for the industry at large. The Ministry of Health has since published guidelines for the

storage and handling of lyophilized spray-on culture products. HACCP (Hazard Analysis Critical Control Points) and biosecurity protocols for cheese plant operations have now been drafted that outline the separation of the farm environment from cheese processing. An evaluation of water sources for cheese plants and dairy farms in general, has been initiated by MAFF. And finally, among all participants in this study, these two cases have generated an increased awareness of the potential for *Listeria* contamination of both cheese and cheese products.

Ed.'s note: our thanks to Josh Waddington, Food Safety and Quality Branch.

An irrigation water project in the Cloverdale area and fecal coliforms:

K. Zimmerman

The Nicomekl River in Surrey supplies water to a series of irrigation ditches in the Cloverdale area. In the summer of 2002, the BC Ministry of Water, Air and Land Protection conducted regular testing of the water in the area and in some instances the water did not meet provincial guidelines for irrigating "ready to eat" crops such as berries and some field vegetables. In particular, the guidelines for *E. coli* and fecal coliforms were sometimes exceeded. This type of contamination indicates that the sources are either human septic systems or livestock manure. From the distribution of the sampling results there appear to be many non-point sources.

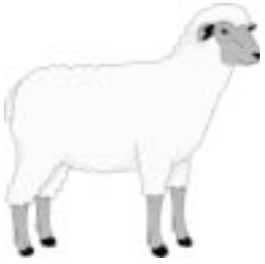
A multi-agency committee headed by BCMAFF is investigating the potential sources of the contamination, and implementing programs for reducing the risk of using the water. Growers are encouraged to:

- leave as many days as possible between the last irrigation and harvest;
- minimize the use of overhead systems for irrigating ready to eat crops; and
- wash produce with potable water before shipping (where applicable).

A landowner contact program in the Cloverdale area will run through February and March, 2003. All landowners and tenants in the area will be personally contacted, to increase awareness of the problem, and to be given information on how they can help to improve the water quality. Livestock producers will be encouraged to double-check their nutrient management programs, and made aware of the upcoming BC Environmental Farm Plans.

– Ed's note: our thanks to Kathleen Zimmerman, Food Safety and Quality Branch,

An unusual abortion storm in sheep:



"This past spring, a group of 600 ewe lambs in western Colorado experienced an unusual abortion outbreak… These lambs were vaccinated against *Campylobacter spp* abortion, BVD, and clostridial diseases. They were bred to lamb earlier than the adult ewes, and were confined to lambing corrals while the ewe bands were still on winter range. At about 90 to 100 days gestation, the owner noticed aborted, very autolyzed fetuses…

"Necropsy and culture of the first of these autolyzed fetuses was unrevealing. A second attempt at diagnosis of more developed, less autolyzed fetuses a week later revealed the presence of swollen livers, scattered hemorrhages in various organs, and the presence of organisms having swimming characteristics typical of *Campylobacter sp* on dark-field examination of abomasal fluid. Bacterial culture of liver and abomasal fluid yielded *Campylobacter coli*.

"Abortions continued over the next few months into the late gestational stage, so an attempt was made to control the abortions by antibiotics. Unfortunately, this organism is slow-growing and not amenable to antibiotic sensitivity testing… with reliability. Accordingly, whole-herd antibiotic therapy was initiated to no effect… Strict sanitation in lambing pens and prompt cleanup of aborted fetuses and fetal membranes likewise had little effect on the disease. In all, approximately 1250 ewes aborted.

"… An autogenous vaccine was prepared from the isolate and will be used on all herd members prior to the 2003 lambing season.

"Campylobacteriosis (Vibrionic abortion) in ewes is usually caused by *Campylobacter fetus subsp fetus*. Infection by this organism is common in unvaccinated sheep and usually results in later-term abortion. This case is unusual in that the causative organism is *Campylobacter coli*, and the disease was occurring at a much earlier stage of pregnancy. According to Prescott, *C. coli* is a normal inhabitant of the intestinal tract of pigs… of concern because of its potential as a cause of gastroenteritis in humans.. source of this outbreak was not identified. Swine operations are few and far between in western Colorado, so it is puzzling as to how this organism occurred on this sheep ranch."

– Ed.'s note: our thanks to author D Schweitzer, *Lab Lines*, newsletter of the Colorado State University Diagnostic Laboratories, vol 7. No. 5, fall 2002.

Worth revisiting - nervous coccidiosis: what is it?

J. Coates

Veterinary practitioners occasionally observe clinical cases of bovine coccidiosis, usually in weaned cattle in late fall or early winter, that also display clinical evidence of nervous signs. Affected animals are usually recumbent, dehydrated, and unwilling or unable to stand. Frank blood mixed with thick mucus may be seen in their stools; necropsy examination reveals evidence of colonic coccidiosis.

The precise nature or etiology of the nervous signs seen remains somewhat enigmatic. A labile neurotoxin (LNT) in the serum of calves has been identified and characterized, but its real significance with regards to clinical signs is poorly understood (Isler et al*).

No significant pathological lesions have been observed in the brain tissue of mice inoculated with serum from calves with "nervous coccidiosis". Evidently calves with coccidiosis that are showing nervous signs are often copper deficient (Jubb, Kennedy and Palmer, Path of Domestic Animals, 1993).

– *Catherine Isler et al. *Characteristics of the labile neurotoxin associated with nervous coccidiosis*. Can J Vet Res 1987; 51: 271 - 276. Reprinted from the Jan/99 *Diagnostic Diary*.

Fatal nitrate toxicity in yearling Holstein heifers:

J. Coates

Three short-yearling dairy heifers from a Lower Mainland farm were submitted to the AHC for necropsy following discussions with the owner and his veterinary practitioner. The history of the case suggested a form of acute feed toxicity. The previous evening, eight yearling heifers had been fed bales of a local grass hay that

had been purchased that day, from another farmer. The animals were all healthy at the time, and had no prior history of illness. Early the next morning, three of the animals were found dead in their pen. The owner immediately removed the hay .

Necropsy findings on the three dead heifers were nonspecific. Extensive congestion of the nasal turbinates and neck vessels was noted, together with tracheal congestion; one animal had limited serosal hemorrhages overlying the greater curvature of the rumen. Rumens were moderately bloated and filled with the suspect hay; no grain was present. Body condition of the animals was poor to fair, with limited heart, mesenteric, and renal fat stores. The blood of the animals was a dark venous colour, clotting appeared poor. A rapid, qualitative nitrate test (performed within the necropsy room) on aqueous fluid taken from the eyes of each of the animals was strongly positive. Quantitative analysis of the hay indicated a nitrate concentration of 3.0%. Plants that accumulate more than 1.5% nitrate in dry matter are potentially toxic (Radostits et al: *Veterinary Medicine*, 9th edition, WB Saunders)

Poorly fed animals as in this case are evidently more susceptible to nitrate/nitrite toxicity than well-fed stock. Other than the level of nitrate in the plant itself, the rate of ingestion of the toxic material is the most important factor influencing susceptibility (Radostits et al: *Veterinary Medicine, 9th ed*). In severe exposures, death may follow within only a few minutes or up to an hour after ingestion of the toxic feed.

The mechanism of action in nitrate toxicity is linked to the conversion of the nitrate to nitrite within the gastrointestinal tract of animals. Absorption of nitrite into the animal's blood circulation results in the conversion of hemoglobin, the oxygen-carrying compound within red blood cells, to methemoglobin. Methemoglobin is incapable of transporting oxygen to the body's tissues. In severe cases as in these heifers, the loss of functional hemoglobin oxygen transport results in acute tissue oxygen deprivation (anoxia), followed by death.

Milder and more chronic exposure levels to nitrates may lead to abortion (levels of 0.5%) in otherwise relatively asymptomatic animals. It's noteworthy that animals clinically ill from nitrate toxicity will respond to intravenous treatment with methylene blue, which rapidly converts methemoglobin back to hemoglobin, restoring its oxygen-carrying capability.

Ruminants likely to be exposed to nitrites or nitrates should receive adequate carbohydrates in their diet. Hungry animals (as in this case) should not be allowed access to feed known to contain high levels of nitrates (Radostitis et al: *Veterinary Medicine*, *9th ed*). With pregnant animals, avoid feeding hay or other feedstuffs that contain even low levels of nitrate, as abortion may result.

Nitrate toxicity as a cause of bovine abortions:

J Coates

Periodically, incidents of bovine abortion are suspect for nitrate abortion. Inevitably, following standard examination and studies of aborted fetal tissues including placenta, there is no evidence of an infectious etiology to these abortions. Widespread placental degeneration, characterized by extensive secondary mineralization, may at times be seen throughout the placental trophoblast, stromal, and vascular tissue, in suspected cases of nitrate toxicity. This change is a nonspecific process, however, and not unique to abortions caused by nitrate toxicity.

Vitreous humour (eye fluid) taken from these calves may be positive for nitrates, if the sample is taken quickly after abortion, and if fetal post mortem decomposition has not

become too advanced. Practitioners are urged to keep this in mind when investigating abortion outbreaks in cattle. An examination of the feed supply of these animals will often indicate a nitrate source such as green feed or oat haylage, etc. Feed samples suspected of containing high levels of nitrates may be analyzed at the AHC.

Diagnosis of nitrate toxicity is usually made in the absence of other factors, infectious or otherwise, that might be linked to the abortions. A comprehensive examination of all fetal tissues, including brain and placenta, is always warranted in these cases, as other factors or agents may also play a role. Low level nitrates may interfere with normal fetal thyroid activity, predisposing the fetus to thyroid hypofunction. Nitrate abortions may occur as a "storm", following a sudden feed change to the offending dietary ingredient. Retained placentas in aborted cows can be anticipated.

When in doubt about the nitrate levels of feed or water sources, send these in for analysis to the AHC. Eye fluid aspirated with an ordinary syringe from the eyes of a dead animal may also prove useful as a rapid, qualitative test in detecting the tell-tale presence of nitrates. A rapid test on eye fluid for detectable levels of nitrates should be useful for at least a few hours after the death of the animal, and depends in large part on the freshness of the carcass. Eye fluid that proves positive is a useful diagnostic finding (see previous article), whereas a negative reading does not necessarily rule out the possibility of nitrate toxicity.

In addition, other signs of nitrate toxicity may be occurring in the cowherd as exposure to feed levels rise. These include acute colic, diarrhea, respiratory distress, and even death, as the levels of hemoglobin conversion to methemoglobin increase. Abortions may thus be one of the earlier signs of a lower level of nitrate toxicity, in which oxygen levels to the developing fetus are significantly reduced.

– *Reprinted from the Jan/99 issue of Diagnostic Diary*

Focus on Staff: Mr Ken Sojonky, Molecular Diagnostics Scientist

Mr Ken Sojonky was recently appointed to the Molecular Diagnostics Section of the Animal Health Centre (AHC), where he conducts research and development of new and existing PCR assays for detection of an extensive range of animal pathogens. Ken enjoys many aspects of his work - not only the various demands generated within a veterinary laboratory setting - but also those arising in the growing and ever-evolving role of molecular biology.

Mr Sojonky brings valuable knowledge and experience to his new role in molecular diagnostics at the AHC. In addition to performing routine PCR testing, it's noteworthy that Ken recently developed a very significant test to differentiate Infectious Bronchitis (IB) poultry viruses. This will allow the tailoring of IB vaccines for individual geographic areas where a particular IB variant virus is predominating. The technique involves the use of a nucleic acid-based sequence detection system. The only other laboratory in Canada performing this test is at the U of Guelph.

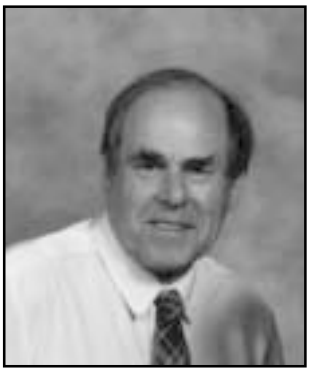
In recent years, Molecular Diagnostics has assumed primary importance within the AHC's diagnostic arsenal. DNA identification of various infectious agents via the polymerase chain reaction (PCR) technique has, without exaggeration, revolutionized the ability of diagnostic laboratories to identify a host of important yet fastidious infectious organisms known to cause infectious disease in animals.

A native of Regina, Saskatchewan, Ken completed his BSc degree in biochemistry from the U. of Victoria. Following graduation he worked as a laboratory instructor in

the Microbiology Department, where he taught undergraduate students. He then worked for three years at the U. of Victoria's Centre for Environmental Health, a cancer research facility, where he studied the mutagenic effects of agents in food and the environment utilizing advanced techniques in molecular biology; these included the polymerase chain reaction (PCR) procedure and nucleic acid sequencing.

Together with his wife and their three children, Mr Sojonky enjoys an active life outside the work environment that includes tennis, skiing, and hiking. We welcome Ken to the Animal Health Centre.

Robert (Bob) Puls retires from the AHC:



Robert Puls recently retired after 34 years in the Toxicology/Clinical Nutrition section of the Animal Health Centre, the longest-serving employee in the laboratory. Originally from England, Bob received a "National Diploma in the Science and Practice of Agriculture" by the National Agricultural Examination Society of England and the Royal Highland and Agricultural Society of Scotland.

Mr Puls immigrated to Canada in 1966. In 1969, following employment at the Ag. Canada Research Station at Saskatoon and later in the Toxicology Section of the Veterinary Diagnostic Laboratory at Guelph, Bob began his BC career at the old provincial Veterinary Laboratory on Gladwin Rd, Abbotsford.

During his tenure at the AHC, Bob demonstrated a primary interest in mineral and vitamin analyses linked to animal nutrition. Perhaps his most significant contribution to agriculture was his publication of the manuals "*Mineral Levels in Animal Health – Diagnostic Data*" and the companion volume "*Vitamin Levels in Animal Health*". Both publications are now in second edition. The reference data in these two books have been widely received throughout North America and indeed, the world.

In recognition of his many years of service, Mr Puls recently received the Commemorative Medal for the Golden Jubilee of Her Majesty Queen Elizabeth II. Bob has two sons, Ken and Brian. Together with his wife Sheila, he is retiring to his small farm near Aldergrove, where he plans to continue his involvement in community projects and raising his sheep. All of us at the AHC wish Bob well in his retirement, and congratulate him on a productive life of sterling service.

What about Pathology?

"Pathology is a changing science, yet its roots remain exceedingly intact. Disease is still often caused by various forms of microbes and toxins, but our continuing fascination with how disease occurs has produced greatly increased levels of resolution and an enriched understanding of the complex mechanisms by which mammals become sick. Paralleling this increased comprehension has been an explosion of detailed information that confounds even purposeful efforts to surround and comprehend it...

"… Disciplinary lines have become blurred. Contemporary pathology is a blend of the disease-oriented aspects of what used to be the intellectual provinces of such diverse disciplines as immunology, cell biology, biochemistry, internal medicine, and physiology… Pathologists have both contributed to and profited from the advances in all these various areas of science…

h:"…The conflict for contemporary pathology lies not in whether the study of lesions is still appropriate but in how best to present lesions in the modern light of what we now know about how those lesions came to be. We must make the lesions come to life..."

– from the Preface of *Mechanisms of Disease: a Textbook of Comparative General Pathology,* 3rd edition, 2002: David O Slauson and Barry J Cooper, authors; Mosby publishers, Toronto, Philadelphia, Sydney, London.

Report on a scientific experiment:

"The investigator reported that one third of the rats were improved on the experimental medication, one third remained the same, and the other one third could not be reported on because that rat got away."

– Edwin Bidwell Wilson (1879-1964): from *More of Mould's Anecdotes*, 1989; Adam Hilger publishers, Bristol and New York.

The secretion of Time:

"… And I felt, as I say, a sensation of weariness and almost terror at the thought that all this length of Time had not only, without interruption, been lived, experienced, secreted by me, that it was my life, was in fact me, but also that I was compelled so long as I was alive to keep it attached to me, that it supported me and that, perched on its giddy summit, I could not myself make a movement without displacing it. A feeling of vertigo seized me as I looked down beneath me, yet within me, as though from a height, which was my own height, of many leagues, at the long series of the years…"

– Marcel Proust (1871-1922), from *Time Regained: in Search of Lost Time*. vol VI; Copyright 1993 by Random House Inc.

The last word:

On Nature:

"Whence our race came, what sorts of limits are set to our power over Nature and to Nature's power over us, to what good we are striving, are the problems which present themselves afresh, with undiminished interest, to every human being born on earth."

– Thomas Henry Huxley, British naturalist, 1863.

On Scientific Method:

"I have a strongly held skepticism about any strongly held beliefs, especially my own."

– Valerie De Lapparent, astronomer.