Animal Health Centre

BRITISH COLUMBIA

Ministry of Agriculture, Food and Fisheries

Diagnostic Diary Vol. 14, No. 2. *The Animal Health Centre of British Columbia: a diagnostic laboratory accredited by the American Association of Veterinary Laboratory Diagnosticians.* **June 2004**

CONTENTS

In this issue:

Editor:

J.W. Coates

Mailing Address:

Ministry of Agriculture,
Food and Fisheries
Animal Health Centre
1767 Angus Campbell Road
Abbotsford, BC V3G 2M3

From the Director

R.J. Lewis:

Highly Pathogenic Avian Influenza diagnosed in British Columbia:

Following receipt of a diagnostic submission from a poultry veterinarian in mid-February, our avian health veterinarian, Dr. Victoria Bowes, suspected the presence of avian influenza virus infection. Rapid evaluation by our molecular diagnostic section confirmed the presence of avian influenza (AI) and the Canadian Food Inspection Agency (CFIA) was immediately notified. The identification of this agent was quickly confirmed by the CFIA as the H7 subtype and further examination indicated it to be the low-pathogenic form of the disease. Since global animal health authorities are planning to make either H5 or H7 subtypes of AI as federally-reportable diseases (regardless of pathogenicity testing), the CFIA quarantined and depopulated this flock of 9,000 birds. As this decision was being made, a younger flock of 9,000 birds on the same farm began showing high mortality and additional samples were taken. The virus from this flock was also H7N3 but had mutated from the low pathogenic form to highly-pathogenic AI.

As of June 08, there have been 42 positive commercial and 11 positive backyard premises in the central Fraser Valley. Each of these has been depopulated plus all commercial and backyard birds within a 3 kilometre radius of the infected premise. All hatcheries have closed and all poultry populations are being shut down either through normal processing activities or active depopulation in the control area. All poultry sectors have been very actively working with the federal and provincial governments to resolve the crisis as quickly as possible. The industry has estimated costs approaching $5 million per day lost due to the outbreak. Significant economic losses apart from the poultry producers include feed companies, processors, equipment dealers, poultry catchers, hatcheries, and others. As well as the Ministry of Agriculture, Food and Fisheries, several other provincial agencies such as the Provincial Emergency Program; Water, Land, and Air Protection; Ministry of Health; and the BC Centre for Disease Control have been actively involved.

Continued Page 2

Highly Pathogenic Avian Influenza diagnosed in British Columbia: *(Continued)*

This is the first case of a List A Foreign Animal Disease in Canada since the foot-and-mouth disease outbreak in cattle in Saskatchewan in 1952. As another form of highly-pathogenic AI due to H5N1 has been concurrent in southeast Asia causing extremely high losses and human fatalities, public health agencies in British Columbia have been very concerned about the ongoing progress of the disease. Since the initial diagnosis and confirmation there have been innumerable press conferences, teleconferences, media reports, and meetings within our ministry and between all of the previously-mentioned agencies.

The National Centre for Foreign Animal Diseases (NCFAD - CFIA reference laboratory) in Winnipeg and the Animal Health Centre have been working together and sharing testing protocols to ensure all samples are tested accurately, rapidly and efficiently. The AHC, in particular the Molecular Diagnostics section, has been testing pre-slaughter samples to ensure only negative birds go for human consumption and the NCFAD has been testing samples from infected premises and those within the 3 km zone. Aggressive surveillance of laying and breeding birds outside the most heavily infected area has been completed. The NCFAD evaluated approximately 11,000 blood samples from this population and, in the same two-week period, the AHC provided PCR analysis for AI on approximately 2,400 tracheal and cloacal swabs.

Preliminary discussions are underway regarding repopulation of infected premises but that can only begin following complete cleaning and disinfection on all infected barns; the tentative date for this to be completed is early June. The poultry industry has opted not to place birds on previously-infected premises for 60 days following approved cleaning and disinfection of the last infected premise. If no additional cases of AI occur within 21 days of cleaning and disinfection then negative premises will be allowed to be repopulated.

The next step is to ensure that full biosecurity precautions are being taken in all the feather industries. The poultry industry has hired two veterinary consultants who have been working with Dr. Bowes, our ministry poultry specialist, and industry representatives to produce a full biosecurity report. This will contain recommendations to prevent a recurrence of avian influenza or any significant poultry disease and the consequent severe economic losses to the industry.

This has been a long process and we hope that our poultry commodity groups will be able to fully recover as quickly as possible. It has been a tremendous effort on the part of all players and I am especially proud of the excellent work that has been done by the AHC staff and the entire Ministry of Agriculture, Food and Fisheries. We hope to take part in a full de-briefing with all players - industry and government (provincial and federal) - once the crisis has been resolved to ensure increasing vigilance and an improved response to any future foreign animal disease outbreak.

From the Animal Health Monitoring Laboratory:

L Curley

Johne's Disease culture and direct fecal exam via PCR:

The Animal Health Monitoring Lab offers BACTEC fecal culture to detect *Mycobacterium avium subspecies paratuberculosis* (MAP). This is a C_{14}-based liquid culture method. Growth of the microorganism gives off radioactive CO2, which is detected by the BACTEC 460 machine. MAP can be detected much faster than traditional culture, often in 2 to 4 weeks. The BACTEC system can identify MAP from a variety of species, including sheep. Negative fecal samples are incubated for a maximum of 8 weeks if submitted fresh, longer if frozen. Fresh samples are much preferred since sensitivity is lost with freezing. Take a minimum of 5 g fresh feces per animal and submit overnight, with ice packs, so that samples arrive at the laboratory as early in the week as possible. BACTEC culture is $30/fecal sample.

Herd screening can also be accomplished by culturing strategic (by age) pools of 5 fecal samples, at $45/pool. Call for more information.

In addition, the Molecular Diagnostics Section offers direct PCR on fecal samples with a turnaround time of 3-5 working days, much faster than culture. However, to date this test has less sensitivity than BACTEC culture. Submit 5 g feces. Direct PCR is $25/fecal sample. *letitia.curley@gems8.gov.bc.ca*

Toxicology news – vitamin D analysis suspended:

D McIntosh

The AHC will no longer provide vitamin D analysis. The cost of maintaining the license to do radioimmunoassay and the high cost of the reagents which have a very short life-span, together with infrequent requests for the analysis, were the reasons leading to this decision. We are attempting to locate another laboratory in North America that offers this service – this information will be reported in the next newsletter.

Short cuts from the Post Mortem Room:

J Coates

Copper toxicosis was diagnosed in a flock of 60 ewes that had experienced sudden and unexpected losses. The veterinarian had observed a yellow, fatty liver on gross necropsy of one of the ewes, together with dark urine. Trace mineral analysis on liver tissue submitted to the AHC indicated copper levels at 548 ppm (wet weight) and kidney copper levels at 32.3 ppm. Microscopic examination of liver revealed chronic-active liver inflammation, bile retention, and degeneration of liver cells. Kidney tissue revealed tubular degeneration and hemoglobinuria (disintegrated or lysed blood cell products within kidney tubules) . The veterinarian was immediately notified of the positive copper toxicity findings.

Cysticercus ovis was tentatively diagnosed by a meat inspection veterinarian in multiple muscle tissue samples taken from several slaughtered feeder lambs. Specimens were submitted to the AHC for additional study. Numerous white, shiny nodules were observed in heart muscle, masseter muscle of the jaw, and in random foci of skeletal muscle.

Microscopic examination of these structures revealed cystic structures consistent with *Cysticercus ovis*, the intermediate stage of the tapeworm *Taenia ovis,* which has the dog (rarely, cats) as its final host. In dogs, the ingested tapeworm cysts in muscle tissue develop into the intestinal form that will later mature and shed eggs. Several of the sections demonstrated viable embryonic tapeworm structures ('protoscolices') within the cysts. Slaughtered lambs that demonstrated the parasite muscle nodules of *T ovis* on veterinary inspection were condemned.

Information was sent to the owner of the lambs regarding the life cycle of this organism, and its direct relation to dogs as final host. Deworming of farm dogs on a regular basis with an effective anti-tapeworm agent was strongly recommended. Dogs should not be fed uncooked portions from any lamb or sheep carcasses as this may only re-infect them with tapeworm cysts embedded in muscle tissue. As far as possible, pasture contamination with dog feces should be avoided since fecal matter may contain tapeworm eggs. Unfortunately, it is theoretically possible that previously exposed wild canids (e.g. coyotes) might also shed eggs from this tapeworm parasite and become a source of infection for sheep or goats at pasture. However, the principal concern remains with farm dogs that carry the adult tapeworm parasite in their gastrointestinal tract.

The ingested eggs of *Taenia ovis* may occasionally infect man as an aberrant host - the intermediate cysticercus stage of *Taenia ovis* as seen in the muscle tissue of these sheep has been reported in the spinal cord of man (*Zoonoses and Communicable Diseases Common to Man and Animals,* 1989: Pan American Health Organization, Sci. Pub No. 503; pp 749-760).

Bacterial abortion in a dairy cow linked to *Bacillus sp*

J Coates

Bacterial abortion caused by *Bacillus sp* was diagnosed in tissues from an aborted seven-month gestation Holstein fetus. A pure culture of *Bacillus sp* was obtained from the lung and stomach contents of this freshly aborted specimen. Despite all efforts, species identification of the *Bacillus* organism in this bovine abortion was not definitively determined. Viral and fungal studies were negative.

Microscopically, the most interesting changes were marked placental inflammation (suppurative placentitis and trophoblast cell necrosis), combined with myriads of tangled Gram-positive bacilli consistent with *Bacillus sp* observed adjacent to and infiltrating placental tissue. Lung tissue revealed a diffuse pneumonia, with similar bacilli observed within lung airways. There was also mild inflammation of the surface of the heart (epicarditis).

Bacillus sp is recognized by Kirkbride and others as a bacterial organism capable of causing bovine abortion (1). In the literature, *Bacillus sp* abortion in cattle has been induced by *B licheniformis,* and in earlier studies by *B cereus* (2). Some strains of *B licheniformis* are thought to have a tropism for the bovine pregnant uterus (2). In addition, *B cereus* and *licheniformis* are occasionally isolated from cattle with mastitis and from horses and cattle with suppurative lesions (3).

There are approximately 22 species of *Bacillus* presently recognized. Most are considered non-pathogenic for animals and have almost no intrinsic invasive ability and multiply only in tissues weakened by injury or other infections – that is, they are opportunistic pathogens (3). According to Kirkbride, the immune reaction at the junction of the maternal and fetal placental compartments is suppressed, most likely to prevent the rejection of the fetus. Consequently, opportunistic microorganisms, even those with lower virulence such as B*acillus sp*, have the ability to multiply and produce lesions at this location resulting in abortion (4).

The most pathogenic *Bacillus* species known is *B anthracis*, the cause of anthrax in animals and humans and immortalized as the infectious agent upon which Koch based his famous postulates (3).

1. Kirkbride CA. Laboratory Diagnosis of Livestock Abortion, 3rd ed 1990;193-194. Iowa State Univ Press, Ames, Iowa.
2. Agerholm JS et al: *Experimental infection of pregnant cows with Bacillus licheniformis bacteria*. *Vet Path* 1999:36;191-201.
3. Timoney at al. *Hagan and Bruner's Microbiology and Infectious Diseases of Domestic Animals*, 8th ed 1988; 206-213.
4. *Bacillus licheniformis* Final Risk Assessment*:* published item no. 3169, Feb 1997. U.S. Environmental Protection Agency.

Blackleg in Fraser Valley beef cattle:

J Coates

Blackleg (bovine clostridial necrotizing myositis), caused by the bacterial agent *Cl chauvoei* was diagnosed on gross necropsy in a pastured four month-old Hereford calf. The diagnosis was confirmed by the Fluorescent Antibody technique (FAT). The owner and veterinarian were immediately notified of the necropsy findings.

At time of submission, the owner stated the calf had shown signs of hind limb lameness the previous day, and was found dead the next morning. The calf was in good body condition and had been thriving. The 'blackleg' lesion was found within the deep adductor muscles on the medial aspect of the right hind leg, immediately adjacent to the femur (hip bone) and just below the hip joint (see Fig.1). Muscle in the affected area was a 'black' color, appeared dry (resembling burnt toast), and had a distinct rancid or butyric odor. The affected muscle on gross examination was approximately 7-10 cm along the medial aspect of the femur, and several

centimeters wide. Microscopically, the lesion revealed an acute, severe, necrotizing, suppurative myositis (bacterial inflammation of muscle tissue).

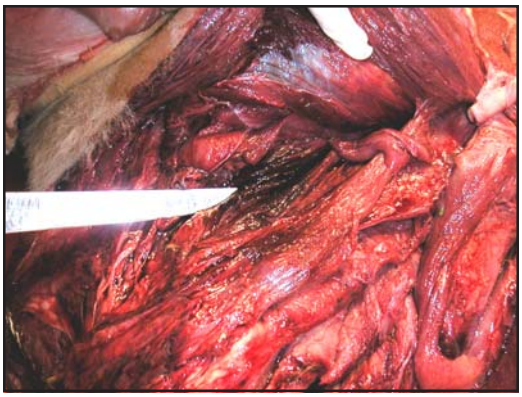

Fig 1. *Blackleg lesion in medial thigh muscle of young beef calf*

In consultation with their veterinarian, the owners immediately vaccinated the remaining young stock with a multivalent clostridial vaccine.

Clostridium chauvoei is the bacterial agent responsible for bovine blackleg. When the disease occurs, it is usual for a number of animals to be affected over a few days. The disease is enzootic in some areas, especially where land is subject to flooding. This animal was from a farm area east of Mission, where there are many low-lying areas subject to seasonal or periodic flooding from the Fraser River and its tributaries.

Although skeletal muscle lesions – such as in this calf – are present in most cases of blackleg, they may be very localized and difficult to locate on routine gross or microscopic examination. However, there is often focal or diffuse fibrinohemorrhagic inflammation of the heart sac (pericarditis) and pleuritis, and where these lesions are present in the absence of severe pneumonia, blackleg should be suspected (1). Unlike *Cl septicum* and other clostridia that may infect cattle, *Cl chauvoei* does not proliferate readily after death, and smears from muscle or other lesions for *Cl chauvoei* can be submitted even from partially autolysed tissues (1).

At time of writing, the owners had not suffered any additional losses.

1. Peter Lusis et al: *Blackleg (Clostridium chauvoei) pericarditis*. In: *Animal Health Newsletter*, Univ of Guelph, Dec 2003: Vol 7, No 4.

Regular monitoring of animal disease continues:

In recent months, the AHC has been actively involved in the diagnosis and epidemiological review of Avian Influenza (AI) virus infection in domestic poultry within the Fraser Valley. In active cooperation with colleagues in the Canadian Food Inspection Agency (CFIA), dedicated AHC staff members including Drs Victoria Bowes and John Robinson and many others, have contributed many long hours in the detection, investigation, and control of this federally reportable avian viral disease.

Avian pathologist Dr Victoria Bowes with
Dr John Robinson, Head of Virology-Molecular Diagnostics

Regular monitoring of animal disease continues: *(Continued)*

Nevertheless, laboratory staff are still confronted with 'everyday' yet urgent animal health concerns of livestock owners from every area of the province –whether it be equine herpesvirus abortion in a group of thoroughbred mares, *Salmonella typhimurium* enteritis in a dairy herd, or copper poisoning (see above) in a flock of sheep.

Significantly, it was this same ongoing daily passive surveillance for animal disease at the AHC that led to the initial diagnosis of Avian Influenza in a Fraser Valley poultry flock.

Each day, week, and month, in multiple animal species from cows to mink to cockatoos, staff of the AHC are called upon to investigate many infectious and non-infectious disease problems. The vast majority are not federally or even provincially reportable diseases, and most are far below the radar screen of public awareness. One typical example reported in this newsletter was a case of 'blackleg' in a herd of BC cattle. For an individual owner confronted with one of these important but lesser known conditions - including blackleg - the provision of a prompt and accurate diagnosis is critical, and remains the personal challenge for each of us at the AHC. *Ed*.

The last word on TSEs:

"Scrapie (in sheep) is the prototype of a group of disease referred to as transmissible spongiform encephalopathies (TSEs). Until relatively recently, the group included the (sheep) disease scrapie, transmissible mink encephalopathy (scrapie infection in mink), and chronic wasting disease that affects mule deer and elk in captivity, and free-ranging mule, white-tailed and black-tailed deer, and elk. Human diseases have included kuru, Creutzfeldt-Jakob disease, and Gerstmann-Scheinker syndrome. More recent additions to this group include a variant form of Creutzfeldt-Jakob disease and fatal familial insomnia in human beings, and bovine spongiform encephalopathy (BSE).

"...With regard to the origins of BSE, epidemiologic evidence strongly suggest that this disease was caused initially (during the 1980s) by the feeding of rations containing meat and bone meal supplements that were contaminated with the scrapie agent.

One of the most intriguing features of the TSEs concerns the uniqueness of their etiologic agents and the manner by which they can be transmitted. The TSEs represent a group of diseases that for the first time in history are caused by agents, unique for each species, that can not only be transmitted as an infectious agent by inoculation of a susceptible animal with infectious tissue (e.g., scrapie infected brain), but can also be transmitted genetically to offspring following spontaneous mutation of the PrP (prion protein) gene in a parent. Such genetic transmissions have been documented in the human diseases Creutzfeldt-Jakob disease, Gerstmann-Sraussler-Scheinker syndrome, and fatal familial insomnia. Creutzfeldt-Jakob disease can also develop spontaneously as a sporadic disease (incidence of 1 per million)… by a mechanism yet unknown, and also... following implantation of tissue from Creutzfeldt-Jakob disease-infected donors.

"…It should be noted that normal cells of the body produce a normal prion protein (PrPc), which is different for each species, and whose normal function is unknown…. PrPc is expressed throughout the body... An abnormal form of this prion protein is present in animals and human beings that develop the characteristic spongiform encephalopathy.

"...When PrPc of one species is inoculated into a different species, the recipient is less readily infected and generally has a prolonged incubation period. This resistance to infection is referred to as the "species barrier..."

– Storts RW and Montgomery DL: *The Nervous System,* in: *Thomson's Special Veterinary Pathology*, 3rd ed., 2001, pp 436-438.

The Last Word

"The important thing is not to stop questioning. Curiosity has its own reason for existing. One cannot help but be in awe when he contemplates the mysteries of eternity, of life, of the marvelous structure of reality."

– Albert Einstein