Animal Health Centre NEWSLETTER

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From The Chief Veterinarian

R. J. Lewis

There are several mileposts in each of our lives that we use to mark major events such as birthdays, graduation, marriage, etc. As most of our lives are spent working, retirement is one of these very important events. Dr. Peter Hewitt will celebrate his retirement from government service on August 20, 1999. Many of you will have met Peter at some point during your association with the Animal Health Center either in his role as pathologist, Chief Veterinarian, or Director of the Animal Industry Branch. Previous to joining the British Columbia government in early 1980, he worked as a pathologist at the diagnostic laboratory in Edmonton for ten years. It has been my privilege to have worked (and partied) with Peter over the last several years. We spent one year at the WCVM in Saskatoon together in the early 1970's where I was just learning the ropes and he was doing a little fine tuning in pathology. Since that time, we have worked together in Alberta and, of course, in British Columbia. (As has been said in relation to other things, "it has been a long, strange journey"!) It has been my great pleasure to have had the opportunity to know Peter both as a friend and colleague and I will miss his sage advice and good sense of humor. I am sure that you will join with me in wishing him all the best for a long and enjoyable retirement.

On the subject of retirements, we are losing another long-time employee, Ms. Olivia Bryant, a valued scientist in our Bacteriology section for 22+ years. She joins her husband in early retirement and will be very much missed by AHC staff and clients alike.

In April, we had the second annual meeting of the AHC Advisory Council which was well attended by representatives from the livestock industry. This meeting provides a good opportunity for Advisory Council members to hear directly of the work being done and to ask questions of AHC staff. We receive very good feedback and advice to assist us in providing a high quality service to all of our clients.

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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

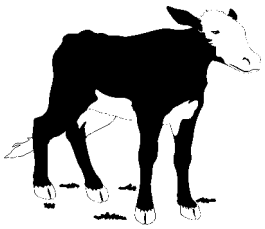
From the Chief Veterinarian (Continued…)

Government is looking at alternate forms of revenue collection in all areas and we have requested permission to accept both Visa and Mastercard as well as debit cards at the AHC. These will provide alternate and convenient methods of payment to all our clientele and we hope to have this in place within the next few months.

Our fee schedule has not been reviewed since September of 1995 and many of our tests must be reviewed and brought more in line with current costs. As we are all aware, costs in general have been increasing gradually (and sometimes not so gradually) over the last several years and the costs of supplies for diagnostic testing have markedly increased. Many of our supplies still come from south of the border and the decreased value of the Canadian dollar has had a very detrimental impact on our costs of doing business. Although we are only in the beginning of making revisions, I hope that there will not be any major increases; however, some charges will definitely have to be increased. It will be a few months yet before these revisions are completed.

Lead poisoning in calves

J.Coates



Lead poisoning in cattle is still occurring regularly in submissions sent to the AHC; this is the second lead-positive submission this spring that this writer has processed.

In this case, a 200 cow herd of beef cattle had suffered mysterious, sudden, deaths in four 2 month-old nursing beef calves in the southern Okanagan. region. All the calves had been found dead. Post mortem investigations by the veterinarian yielded little on examination, other than red, congested lungs, and tracheal froth. Fixed and fresh specimens from one of the calves yielded no evidence of infectious disease.

Lead levels in liver taken from the submitted animal were measured at 17ppm., and kidney levels at 46 ppm. These levels are diagnostic for lead toxicity.

Although the owner inspected the area in which the calf was found, no source of the lead found in this calf was determined. However, the pasture included an old rodeo ground, and possibly a battery, or other lead-containing castoffs had been uncovered by the animal. It is not known whether the other 3 calves also died from lead toxicity, as no samples had been taken.

There are probably a number of lead toxicity cases in the field that are never seen by a practitioner, and never reported. Although lead has been removed from petroleum products for some time now, lead poisonings still occur. Discarded, used batteries found in old pasture refuse dumps, or lying along fence lines, for example, are one of the primary sources of this toxic compound for cattle. Both liver and kidney are important for the detection of lead in animal tissues.

On Eccentricity:

"…In this age, the mere example of nonconformity, the mere refusal to bend the knee to custom, is itself a service. Precisely because the tyranny of opinion is such as to make eccentricity a reproach, it is desirable, in order to break through that tyranny, that people should be eccentric. Eccentricity has always abounded when and where strength of character has abounded; and the amount of eccentricity in a society has generally been proportional to the amount of genius, mental vigor, and moral courage which it contained. That so few now dare to be eccentric marks the chief danger of the time…"

— John Stuart Mill (1806-1873); taken from his essay, *On Liberty*.

Verotoxin positive hemolytic *E coli* cultured from neonatal calves

- *J Coates, J Greenfield*

Bacterial cultures of 7 day-old calves ill with a bloody diarrhea, taken from feces prior to treatment, yielded a heavy growth of cytotoxin positive hemolytic *E.coli*.

Gut contents from these neonatal calves also yielded bovine coronavirus. Bacterial cultures from intestinal tissues on a dead calf that had been extensively treated yielded only *Pseudomonas aeruginosa*.

This is one of the first cases of verotoxigenic *E.coli* cultures that this laboratory has identified. Coliform plates were submitted to Vancouver Public Health laboratories for identification of the verocytotoxin. *E. coli* strains were both K88 and K99 negative.

These toxins are referred to as verotoxins since they are capable of killing Vero cells *in vitro* (i.e., on cell culture).

A few strains of *E.coli* O serogroups have been clearly associated with a hemorrhagic colitis in calves (Schoonderwoerd et al, 1988), but comprehensive identification of serogroups is rarely undertaken today in animals or man. Several studies have shown an association between diarrhea in calves over 3 weeks of age and the presence of verotoxigenic E. coli in the feces (*Pathogenesis of Bacterial Infections*, Gyles and Thoen, 1991).

These verotoxin positive, enterotoxigenic strains of *E. coli* not only cause diarrhea and secondary malabsorption of nutrients and electrolytes, as well as damage to intestinal mucosal cells, but may also produce an erosive fibrinohemorrhagic enteritis (Jubb, Kennedey, and Palmer, *Pathology of Domestic Animals*, 1993).

After antibiotic treatment, submitted intestinal contents from the calf yielded a heavy growth of *Pseudomonas aeruginosa*. This antibiotic-resistant organism will frequently proliferate in neonatal calf intestinal tracts where antibiotic usage has been extensive, and, as a result, may often be the only surviving organism grown on culture.

Goiter in day-old calves

J.Coates

Goiter was diagnosed clinically by the veterinary practitioner in a group of neonatal beef calves. Clinically, 2 of the first 7 calves born early in the spring were born alive but weak, were unable to stand, and bawled constantly. The throat area was prominently swollen in the calf from which fixed and fresh tissues were received at the AHC for further examination.

Microscopic examination of thyroid tissue from the affected calf showed severe hyperplasia (hyperactivity) of the glandular tissue. Unfortunately, a blood serum sample is needed to measure iodine, and could not be done on the fresh tissues submitted. Nevertheless, trace mineral analysis was done on liver tissue, and selenium liver levels were deficient at 0.19 ppm ; adequate Se levels for a bovine newborn vary from 0.30-1.20 ppm (Puls: *Mineral Levels in Animal Health*, 1994). This information was immediately relayed back to the practitioner.

Iodine deficiency should always be considered as a possible contributing factor or differential diagnosis where there are ongoing problems with weak, nonviable, or stillborn calves. The prominently enlarged thyroid gland as described in this case is

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Goiter in day-old calves (Continued…)

not always readily determined clinically or even on gross post mortem; microscopic examination of tissue is recommended where the condition is suspected, as was done in this case. In newborn calves, a thyroid with a total weight of over 30 g is likely to be abnormal, while the histological appearance of thyroid glands weighing less than 30 g cannot be predicted from their weight (Smyth et al; *Stillbirth/perinatal weak calf syndrome: a study of calves with an abnormal thyroid gland*. Vet Rec 1996; 139: 11-16).

In the vast majority of cases, thyroid enlargement in the neonatal calf is due to a simple deficiency of the trace element iodine within the ration of the pregnant cow. Frequently, as was noted in this case, simple iodine deficiency is only the tip of the iceberg; other nutritional deficiencies are also very likely present. In this case, selenium deficiency was also present, although microscopically there was no overt evidence of heart or skeletal muscle damage linked to this element (White muscle disease). Where there are obvious problems with iodine deficiency, an over-all ration review is worthwhile.

It is probably a good idea not to rely solely on iodized salt blocks as a source of iodine for a herd. Individual intake of iodine from these salt licks likely varies greatly. Weathering and sublimation of iodine from the blocks may reduce the actual levels of iodine being offered to the pregnant cow. It is better to offer the trace element iodine and other trace elements such as selenium, within the ration, per se.

One of the problems with iodine deficient calves, as described here, is that they are very weak, and often unable to stand, Some will show nervous signs as described here, suggestive of brain injury of uncertain cause. Lungs may be poorly inflated in iodine deficient calves due to a lack of surfactant, a substance needed to encourage lung inflation after birth. Brain damage can also be anticipated in some of the calves born alive but with signs of iodine deficiency.

Note that in some instances elevated nitrate levels in drinking water in feed may also contribute indirectly to goiter in neonatal calves, by interfering with the normal integration of iodine into thyroid hormone (thyroxine) production.

Listerial encephalitis in a 6 week-old lamb

J. Coates

A lamb was submitted for necropsy after briefly demonstrating clinical signs referable to the central nervous system; depression, paddling, recumbency, and finally death. Examination of brain tissue indicated a mixed cellular meningoencephalitis within brain tissue, with the significant lesions or injured areas located within the medulla, and the overlying white matter tracts of the cerebellum.

Microscopically, perivascular cuffing of vessels was marked and widespread in these areas, together with microabscessation, edema (spongiosis) of the neural tissue, and necrosis of neurons. The location and microscopic characteristics of the brain lesions are typical for listerial encephalitis of sheep, caused by the bacterial organism *Listeria monocytogenes*. The organism was cultured from the brain tissue of the lamb, confirming the diagnosis.

For the veterinary practitioner, one of the interesting points to keep in mind when attempting to diagnose (or rule out) this bacterial disease in a variety of domestic

Continued Page five…

Listerial encephalitis in a 6 week-old lamb (Continued…)

J. Coates

animals is that the lesions tend to be restricted to the cerebellar white matter tracts and, more especially, the underlying neural tissue of the medulla and proximal cervical cord. If only portions of cerebral cortex or midbrain are submitted, it is quite possible that the site of diagnostic lesions will be missed entirely. This is also true for bacterial culture; specimens sent in for culture should always include the medullary tissue, and adjacent white matter tracts of the cerebellum.

To satisfy the needs for both microscopy and bacterial culture, the most reasonable approach is to cut the brain lengthwise along the midline. One half is submitted for microscopy, and one-half for culture. One of the differential diagnoses to listerial encephalitis in sheep is polioencephalomalacia, a laminar degeneration of cerebral cortical neurons usually linked to thiamin deficiency. Microscopically, lesions are often most prominent in the occipital cortex. By sending in one half of the entire brain fixed, for possible microscopic detection of *Listeria* sp., it is still possible, if required, to examine for polioencephalomalacia, as well.

Listerial encephalitis is not restricted to sheep, but may also be found in cattle, goats, camelids, and potentially in pigs. Generally 3 syndromes are associated with the organism; abortion, bacterial septicemia, or bacterial encephalitis, as in this animal. The causative bacterial agent, *L. monocytogenes*, is ubiquitous in nature, and in animal and human feces. It survives for years in soil, milk, silage, and fecal matter. It is interesting that the organism is more heat-tolerant than most other non-sporulating bacteria and has been shown to survive pasteurization by the low-temperature holding process (*Hagan and Bruner's Microbiology and Infectious Disease of Domestic Animals*, 1987).

There is no vaccination available to prevent listeriosis in domestic animals.

Herpesvirus mammillitis in dairy heifers

J. Coates

A veterinary practitioner sent in teat swabs taken from a first lactation dairy heifer that had developed ulcerative teat lesions. Mastitis followed.

The veterinarian provided a good clinical history, in which evidently first lactation animals on this particular farm would develop multiple vesicles up to ½ cm diameter on the skin of usually only one teat. These would later rupture, forming scabs. Mastitis invariably resulted in that quarter, and often use of the quarter was eventually lost. One to 3 heifers were usually involved each year, and in recent years about 10 animals had been affected to date. *Actinomyces pyogenes*, an opportunistic pathogen, was also cultured from the teat swabs.

Viral studies on the submitted samples were positive for a bovine herpes virus on electron microscopy. This was presumed to be bovine herpes virus 2, the causative agent of bovine herpes virus mammillitis.

The exact mode of transmission of bovine herpes mammillitis is unclear, because the disease is usually seasonal, and exceptional efforts at hygiene and quarantine fail to curb the spread of the condition within a herd. Insects are believed to be involved as vectors of the virus (Scott: *Large Animal Dermatology*, 1988), although it is also believed that some cattle become virus carriers and, under stress, shed the virus. The incubation period is 3 - 7 days.

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Herpesvirus mammillitis in dairy heifers (Continued…)

As described in this case, this viral teat disease of cattle tends to affect heifers more than mature cows. The disease is sudden in onset, with teats becoming swollen and tender. Vesicles may appear in a few hours but, in many cases, the epithelium simply sloughs and exposes inflamed subcutaneous tissue. Copious exuding of serous fluid and crust formation then follows. According to Scott, the number of affected animals at any one time varies widely, but mortality is negligible. Nevertheless, economic losses may be very significant as a result of decreased milk production and an increased incidence of mastitis.

In British Columbia, the significant differential diagnoses in this condition are pseudocowpox, udder impetigo due to *Staph aureus* infection, teat fibropapillomas, and so-called black pox (due to poor milking machine technique). True cowpox is very rare (Radostits et al: *Veterinary Medicine*, 1994).

This laboratory identifies herpes virus mammillitis only rarely from submitted samples. The true incidence of the condition among B.C. dairy cattle is probably higher than our records here would indicate. It is a condition that veterinary practitioners and dairy operators might keep in mind when confronted with recurring superficial sores on cows' teats.

Short cuts from the post mortem room

— A 6 week-old lamb was brought in for necropsy following progressive weakness and terminal recumbency that extended over 3 or 4 days. The lamb had been seen earlier staggering in from the pasture, with other animals in the flock. The animal was euthanized. Post mortem revealed **extensive predator-origin lacerations** of the subcutaneous tissues over the first three cervical vertebrae, with several foci of vertebral crushing, splintering, and fracturing. Areas of spinal cord were exposed. The area was very septic, emitting a foul smell. Puncture wounds were readily seen on the underlying surface of the skin. Interestingly, there were no readily detectable outer, or externally visible evidence of the severe neck injuries. **Coyotes** had been seen in the pastures of the sheep, and it is likely that this lamb was attacked by a coyote, but somehow managed to escape.

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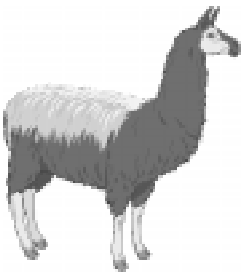
— Portions of oviduct from a laying hen were submitted by a practitioner, for examination. Gross and microscopic examination of submitted oviduct tissue indicated chronic inflammation (salpingitis), with mucosal and luminal accumulations of cheese-like (caseous), necrotic material. Coliform bacteria were detected on culture, including *E.coli*; *Actinobacillus salpingitidis*, an organism of doubtful pathogenicity, was also detected.

Inflammation of the oviduct is caused by a great variety of infectious agents. In many cases, bacteria from the intestinal tract and cloacal area ascend into the oviduct. Air sac infections (particularly the abdominal air sac) with *E. coli* may also lead to oviduct inflammation, or salpingitis. At the AHC, salpingitis is seen regularly in culled laying hens, or hens found dead and brought in for necropsy. Salpingitis is a common cause of sporadic mortality in mature laying hens.



— As an incidental finding, **whipworms, (*Trichuris sp*)**, were found in mild to moderate numbers within the descending colon of a mature llama that had died of a severe fibrinous peritonitis caused by leakage of intestinal contents. In larger concentrations, this organism may contribute to anemia, malabsorption of fluids and nutrients, and chronic inflammation of the caecum and colon. The writer has observed this helminth in large numbers within the caecum and spiral colon in individual grower or feeder pigs that have been raised outside; the animals are frequently emaciated and unthrifty, and anemic, due to heavy *Trichuris* infections. Similar cases have occasionally been seen in young feeder or weaner calves.

* * * * *

— **Acute copper toxicity** was observed in a mature holstein cow. Liver copper levels were measured at 491 ppm., and kidney copper levels at 52 ppm. On gross necropsy, kidneys of the animal were almost black in color, and very swollen. Microscopically, there was severe hemoglobinuria; chronic inflammation of the liver with bile duct hyperplasia was also present, together with acute degeneration and necrosis of centrolobular hepatic cells. Examination of all dietary sources of copper was immediately recommended.

* * * * *

— **Hemolytic *Staphylococcus aureus*** was cultured from milk samples from fur-bearing Blue fox vixens that were not allowing their young to nurse. Some puppies were being killed or severely traumatized by the vixens. Nestbox sanitation was in need of improvement. None of the puppies had nursed. Vitamin A levels in a composite liver sample from 4 of these day old puppies was 8 mcg/g; liver selenium levels were measured at 0.27 ppm. and Cu levels at 6.2 ppm. Tests for canine viruses including canine distemper virus, and herpesvirus, were negative. Hemolytic *staph aureus* was also cultured form the organs of some of the puppies.

Where sanitation is poor and *Staph aureus* is present, bacterial contamination of fox pups within the birth canal of the vixen may occur even before they are born. Improvements in sanitation have been urgently recommended; acute treatments to control the problem have been undertaken by the veterinary practitioner; a review of dietary vitamin-trace mineral supplements is also recommended. As a general rule, lactating fox vixens require a diet of 33 % protein, and 14% fat. In domestic dogs, puppy liver vitamin A levels tend to reflect those of the dam (Puls, 1994, Vitamin Levels in Animal Health).

It was also recommended that the pelleted feed of these fox vixens (they were also fed the viscera of freshly killed chickens) be analyzed for protein, fat, as well as vitamins A,D, and E, and essential trace elements such as Se, Cu, and Mn. The owner had previously been obliged to purchase the pelleted feed in large quantities; it is possible that vitamin activity in the pellets may have deteriorated following storage for long periods prior to feeding to the pregnant females.

* * * * *

— Severe **inflammation of the intestine** (fibrinoexudative, necrotizing enterocolitis) was diagnosed in a 4 year-old **pronghorn antelope** on necropsy, following a short period of illness. A heavy growth of *Clostridium perfringens* was cultured form the intestine. Toxicological analysis of liver tissue also indicated the animal was low to deficient in copper, with levels recorded at 7.2 ppm. The animal was not from the wild, but had been kept at a game farm. In the wild, pronghorn antelopes are well adapted to a diet consisting almost entirely of roughage. However, they are often susceptible to disruptions of the gut microflora (dysbiosis) when they have access to prepared rations that contain higher quantities of grain or carbohydrate.

Hydatid cysts of *Echinococcus granulosus* in moose tissues

J. Coates

Specimens of fresh frozen moose liver were received from an area in northwestern British Columbia. The animal had been shot in the wild. On cleaning the carcass, white, round cysts varying from 1 to 3 cm or more in diameter were noticed within the liver tissue, most of them readily apparent as fibrous white cysts bulging slightly from the liver capsular surface.

A second case was recently received from the same general area of B.C.; cystic structures observed by a hunter in lung tissue taken from a killed moose were later identified at the AHC as hydatid cysts of *E. granulosus*.

Microscopic examination of the cysts revealed numerous tapeworm larval structures called **protoscolices** floating within cyst fluid. These cystic structures were consistent with the **intermediate-host (hydatid) form** of the tapeworm ***Echinococcus granulosus***. In its mature state, this parasite utilizes wild canids such as the wolf as well as domestic dogs as its final (definitive) host, and resides within the animal's intestinal tract.

In the wild (or sylvatic) cycle, a wolf that is infected by this intestinal tapeworm excretes the eggs of the parasite in its feces. Browsing moose inadvertently ingest the tapeworm eggs, which then hatch and migrate in a larval form from the intestinal tract of the moose to various abdominal viscera, such as liver and lung. There the intermediate stage of the parasite develops, forming the large, fluid filled cysts, as seen in this submitted liver sample. The cycle of the parasite is completed when the moose is killed and eaten by wolves. Upon ingestion, the parasite once again enters the predator's intestinal tract and then develops into a mature, egg-shedding tapeworm. In most situations, these cysts are not harmful to the well-being of "normal" intermediate hosts.

The cysts of the tapeworm observed in infected moose viscera are not infectious for humans, although esthetically they are unpleasant. However, these cysts of *E. granulosus* are infectious to wild and domestic canids as mentioned earlier. **For this reason, the pluck or viscera of wild animal carcasses that contain these cysts should be incinerated. They should never be fed to dogs**. They should not be buried, since scavengers may later dig them up, and become infected shedders after eating the contaminated tissues.

Humans are susceptible to becoming infected with the parasite if tapeworm eggs found in canid feces are accidentally ingested; in these instances, tapeworm cysts may develop in the visceral or other tissues of humans, just as they do in the natural, herbivorous, wild hosts. Hydatid disease caused by the intermediate larval form of *E. granulosus* is thus a potential zoonosis for man; that is, it can occur in humans that have accidentally ingested tapeworm eggs excreted by infected canids, either wild or domestic.

– reference:
Hydatidosis. In: *Zoonoses and Communicable Disease Common to Man and Animals,*
2nd edition,1989: 716 - 736. Published by Pan American Health Organ.

Other domestic species that may also become infected as intermediate hosts with *E. granulosus* include sheep, cattle, swine. goats and camelids. In Australia, three strains of *E. granulosus* are distinguished; one circulates between the dingo and marsupials such as wallabies and kangaroos, while the other two circulate between dogs and sheep.

Similar, related organisms that also cause hydatid disease in intermediate hosts are *Echinococcus multilocularis*, *E. oligarthus*, and *E.vogeli*. *E. multilocularis* use the arctic and red foxes as definitive hosts, with several wild rodents as intermediate hosts; this tapeworm is reported in foxes in the northern hemisphere, including several provinces in Canada. Domestic dogs and cats may also enter the cycle of this parasite by feeding on infected wild rodents. Infections by *E. granulosus* and *E. multilocularis* can thus both exist in the same areas of Canada, though with different intermediate hosts. *E. oligarthus* and *E. vogeli* are present only in South and Central America.

Zoonotic concerns involving wild beavers in B.C.

S. Raverty

A beaver was recently found dead along a trapline in northern British Columbia. The carcass was opened and the internal organs were inspected by a number of lay individuals. An unknown disease was suspected due to extensive bleeding throughout the subcutaneous tissue, abundant gelatinous material within the abdominal cavity, and an abnormally appearing liver. A local public health nurse was notified. The carcass was then frozen, and forwarded to the AHC for post mortem evaluation.

The most significant lesions were pronounced enlargement of the liver and spleen where there were disseminated, focal areas of tissue degeneration (necrosis).

Histopathology disclosed severe necrosis and inflammation (hepatitis) of the liver. Rare, degenerate, predominantly intracellular, gram negative coccobacilli were observed within the liver tissue, and although aerobic culture failed to yield any significant pathogens, a bacterial etiology was a strong consideration.

Primary differential diagnoses for similar tissue changes (lesions) in beaver include tularemia (*Francisella tularensis*), pseudotuberculosis (*Yersinia pseudotuberculosis*), salmonellosis (*Salmonella sp*), and plague (*Yersinia pestis*). All these agents may pose a significant health threat to exposed individuals as they are zoonotic (capable of spreading from animals and causing disease in humans).

Due to the extent of liver and splenic involvement, lack of bacterial growth (possibly related to the freezing and thawing of the carcass), and history of human exposure, representative wax blocks of preserved tissue were forwarded to the Prairie Diagnostic Services at the Western College of Veterinary Medicine, Saskatoon, for immunohistochemistry. Positive antigen for tularemia (*Francisella tularensis*) was demonstrated in multiple inflammatory foci within the liver.

Francisella tularensis is a recognized bacterial pathogen of beavers. It is also commonly isolated from rabbits, rodents, and other wild animals and can be recovered from the environment, including mud and water. Incidental infections are recognised in dogs, cats and humans. Infection may occur through ingestion of the organism, or by contamination or inoculation of the skin. In humans, most disease reports of this zoonosis arise from contact with infected rabbits, where exposure has occurred during the skinning process. Symptoms may vary. These include cutaneous ulcers of the hands or fingers; eye involvement (through contact with contaminated hands); lymph node enlargement; abdominal pain, or fever.

Within days of the initial presentation of this animal, another mature beaver from the lower mainland was found dead and was presented to the AHC for evaluation. Gross lesions were comparable to the case just described. However, bacterial culture of the liver yielded a heavy growth of *Yersinia pseudotuberculosis*. Due to public health concerns, a veterinarian or conservation officer should be contacted whenever a severely ill or dead beaver is found. The animal should be left intact. In populated areas, or where there has been human exposure, the animal should be presented for post mortem examination. The threat of zoonotic transmission of pathogens from beavers to man should not be underestimated.

Northern Dancer, a famous Canadian (horse):

"…On 2 May 1964 Northern Dancer won the Kentucky Derby, the most famous race in the world. He was the first Canadian to win the race, and no one had ever raced it faster. He won the Preakness, another big race…Northern Dancer had… many children… Northfield, True North, and Najinsky were just some of his children that were great racers. But none of them was as fast as Northern Dancer."

– from the book *The Canadian 100. The 100 Most Influential Canadians of the 20th Century,* by H. Graham Rawlinson and J.L. Granatstein; McArthur and Co publishers, Toronto, 1997.

Sturgeon Mortalities along the Harrison River, British Columbia

- *Stephen Raverty (AHC) and Lee Nikl (Fisheries and Oceans Canada)*

S. Raverty

During late May and early June, 1999, several anglers who frequent the Harrison River reported mortalities of wild, subadult white sturgeons (*Acipenser transmontanus*). A single dead sturgeon was also found in Harrison Lake. The Harrison River is a clear water river which flows from Harrison Lake, British Columbia, and joins with the Fraser River. The Harrison River has a gross drainage area of approximately 7870 km^2 and daily average June flow of 936 m^3/s. The system is an important producer of chinook (*Oncorhynchus tshawytscha*), sockeye (*O. nerka*), and other salmon species. It also supports an important recreational fishery for white sturgeon. An investigation into these sturgeon mortalities was undertaken jointly by the federal Department of Fisheries and Oceans, and the provincial Ministry of Environment and Ministry of Agriculture and Food.

On an initial survey of the river, June 10, 1999, 6 dead sturgeons were identified by field officers. A compilation of subsequent reports from several anglers and fishing guides placed estimated mortalities between 10 to 12 fish. However, the Harrison River has several very deep pools; in addition, at the time of the incident, there were a number of flooded islands where additional mortalities may have escaped detection. Two of the initial 6 fish were collected and presented to the Animal Health Center for evaluation. Samples of the remaining dead sturgeons were not submitted, either because they were too deep within the water column for retrieval, or because of advanced post-mortem decomposition.

On examination, the two sturgeons were subadults, in good flesh, and in suitable condition for post mortem. In the smaller of the two fish (length 160 cm, body weight 21.4 kg), there was abundant blood tinged abdominal fluid (serosanguinous ascites), as well as an enlarged spleen and ulcerative colitis. The larger fish (length 231.1 cm, body weight 72.5 kg), aside from a small number of encapsulated, peritoneal (abdominal cavity) nematodes, exhibited no significant gross lesions.

Histopathology disclosed subacute inflammation of the spleen. Lesions consistent with bacterial gill disease were also noted. Aerobic bacterial culture yielded moderate to heavy growth of *Aeromonas hydrophila* from multiple tissues in both fish. The polymerase chain reaction procedure (PCR) was negative for infectious hematopoietic virus (IHNV). Trace mineral analyses were within normal limits. Selected tissues have been forwarded to the Institute of Ocean Sciences, Sidney, B.C., for analysis of dioxins, furans, and selected PCB congeners.

The Harrison River watershed has few stressors and is sparsely populated; there is little industry with the exception of tourism at the village of Harrison Hot Springs, and some logging activity in the higher reaches of the watershed. There is a secondary-treated sewage outfall located near the outflow of Harrison Lake; this facility and its records were inspected. No evidence was obtained of process upsets or plant malfunctions at the time of the mortalities or for the month antecedent to the epizootic (an epizootic is an infectious disease, frequently widely diffused and rapidly spreading, which is only occasionally present in an animal community). Water Quality testing carried out on the Harrison River and Harrison Lake indicated slightly supersaturated oxygen levels, and a pH approaching neutral; water temperatures in the Harrison River ranged from 13^0C to 16^0C at various locations. These findings are

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considered unremarkable, and the slight oxygen supersaturation is attributed to photosynthetic activity of aquatic macrophytes along the bottom of the river.

At the turn of the century, the sturgeon fishery was a burgeoning industry with up to 100,000 kg. of fish harvested per year. However, through over fishing and other factors, the stock quickly collapsed. Currently, only a catch and release sport fishery is practised, with retention of caught fish prohibited.

At present, there is still a profound lack of understanding of wild sturgeon diseases. Whenever mortalities occur, efforts are made to identify potential pathogens and possible contributory, environmental factors. In this case, although the proximate cause of death of the smaller sturgeon could be attributed to septicemia, there were still no identifiable environmental or host factors which may have predisposed this animal to infection.

The diseases in these stocks likely reflect complex and dynamic processes which are still only superficially understood. Future work to further define sturgeon pathogens and the development of diagnostic protocols for investigations, will contribute significantly to resolving the epizootiology of these outbreaks.

Cutaneous mycosis in dwarf aquatic frogs (*Hymenochirus boettgeri*):

– Stephen Raverty
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Three of 10 dead dwarf aquatic frogs (*Hymenochirus boettgeri*) were recently presented by a local hobbyist to the Animal Health Center for diagnostic evaluation. The frogs had been procured from a wholesaler in Delta, B.C., in September 1998, having originally been imported from Hong Kong. Two mortalities had occurrred shortly after arrival. The initial loss of these 2 frogs was presumptively attributed to stress of transport and adjustments to new surroundings and conditions. All 10 frogs were housed in a glass aquarium with a silica sand substrate, a corner charcoal filter, and a small number of aquatic plants consisting of grasses and floating frogbit, or duckweed. The animals were maintained on a diet of artemia and thawed, frozen blood worms. Approximately 50% of the water was routinely changed weekly. There was no history of clinical disease and the animals had repeatedly exhibited mating activity. Over the course of 7 days in May, 1999, all ten died acutely with no apparent clinical signs.

The frogs were presented formalin-fixed for necropsy examination. Apart from variably extensive opacity of the skin that was associated with excessive mucus secretion, there were no apparent lesions.

Microscopically, throughout the ventral and to a much lesser extent the dorsal aspect of the body, there were variably extensive regions of thickening within the superficial corneal layers of the skin (orthokeratotic hyperkeratosis). These regions were intermittently overlaid by small aggregates of predominantly extracellular bacterial rods, or bacilli. There were numerous, discrete structures (zoosporangia) interspersed within varying levels of the superficially thickened and occasionally hyperplastic skin epithelium; these structures contained multiple, 2-3 *u*m, round to oval basophilic spores. The microscopic lesions and fungal organisms were compatible with cutaneous chytridiomycosis (a fungus from the phylum Chytridiomycota). This is an emerging condition associated with epizootics in wild and captive frog populations.

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Cutaneous mycosis in dwarf aquatic frogs (*Hymenochirus boettgeri*) (Continued…)

This fungal organism was initially recognized as a pathogen of rainforest frogs in Australia in 1993, and has subsequently been associated with massive die-offs of wild frogs in Panama, in 1997. More recently, the organism was identified in captive poison dart frogs (*Dendrobates* spp) and White's tree frogs (*Litoria caerulea*) that are kept at the National Zoo in Washington, D.C. Historically, the organism had been isolated from plants and insects, with no reported cases of vertebrate infections.

As the skin of frogs is important for normal respiration and maintaining body acid-base balance, the proximate cause of death of these animals has been postulated to have involved impaired gas and ion exchange through the skin. Because specialized media is required to culture this fungal organism, the most reliable means of diagnosis is by histopathology of representative skin lesions. Prime differentials in this case would include *Saprolegnia* spp and chromomycosis (*Phoma* spp or *Phialophora* spp), which may be readily distinguished by hyphal morphology.

In contrast to previous reports, this presentation is unusual in that the dwarf aquatic frog is distantly related to previously reported, affected species, and is an (entirely) aquatic species (non-terrestial). Because this frog collection was maintained separately from other stock (from which no fungal infections had been previously recognized) and fed frozen insect larvae, the animals were likely infected subclinically at the time of acquisition, with losses precipitated by some other process. Attempts to ascertain if these frogs were captive-bred in Hong Kong, or had been captured and imported from Africa, are underway.

This organism is considered an important, emerging pathogen of wild and captive frog species, and reinforces the need for appropriate quarantine and diagnostic evaluation of all recently acquired animals.

Vacuolar myelinopathy in Atlantic salmon fingerlings

– Stephen Raverty (AHC) and John Brocklebank (Brocklebank Mobile Services)

Atlantic salmon fingerlings, weighing between 7-9 gm., were presented for diagnostic evaluation; the fish were in good flesh with no apparent lesions.

The fish were from a hatchery maintained by a ground water source. Historically, fish from the hatchery had annual problems of recurring listlessness, lethargy, darkening skin, and inappetance. These clinical signs were often precipitated by rapid and profound drops in environmental water temperatures at the hatchery during the latter part of winter and early spring. Although morbidity (numbers of fish actually ill) was high, few mortalities were incurred; fish either spontaneously recovered or anecdotally improved following supplementation of a commercial starter feed with a vitamin premix, for 5 days.

Microscopic examination of tissues was limited to the central nervous system, where there were severe degenerative changes within the spinal cord (vacuolar myelinopathy), with scattered macrophages filled with engulfed (phagocytized) myelin. No other microscopic lesions were seen in examined tissues. Within white matter tracts of the medullary area of the brain, there were clusters of vacuoles of varying sizes compatible with intramyelinic edema; these were separated by fine, myelin strands.

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Vacuolar myelinopathy in Atlantic salmon fingerlings (Continued…)

To our knowledge, this condition has not been previously recognized in fish. Based on extrapolation from mammalian and avian species, primary differentials to be considered are nutritional, environmental, or other factors. For example, heavy metals such as lead, arsenic, mercury and copper, as well as various other substances including inorganic tin, tributyltin, and strychnine, have been associated with similar disease processes in higher vertebrates. Abnormal levels of vitamins A, B and E have also been reported to induce similar lesions in mammals, as has possible exposure to algal toxins such as microcystin, saxitoxin, or adventitious toxins such as fumonsin.

Trace mineral analysis of fish samples yielded levels of the above metals within normal, in-house reference ranges; chemical analysis of selected water samples revealed no toxins. Efforts are currently underway to monitor environmental parameters and to better assess the nutritional status of the brood stock and developing progeny.

The condition appears to have resolved with no significant impact on subsequent ongrowing stages.

Pilchard (*Sardinops sagax*) die-off along B.C. coast

- J. Constantine, J. Robinson, and S. Raverty.

In November/December 1998, large numbers (hundreds of tonnes) of pilchard fish (*Sardinops sagax*, commonly known as the Pacific sardine) were found shoaling and dying in the bays and coves from Johnstone Strait to Queen Charlotte Strait at the northern tip of Vancouver Island. Scientists from the federal Department of Fisheries and Oceans (DFO) investigated the losses and reported that the pilchards were dying from a North American variant of viral hemorrhagic septicemia (VHS). Fish farmers expressed concern about the potential for this condition, as well as other diseases carried by the pilchards, which may be transmitted to salmon farmed in the area. In response, the British Columbia Ministry of Agriculture and Food (MAF) staff collected samples of pilchards for diagnostic evaluation.

On Jan 13, 1999, 14 terminally ill fish were collected from Beaver Cove, Vancouver Island. The samples were taken opportunistically (i.e., by a 30-foot dip net from the side of a 1ive-haul well boat) as weather conditions prevented more rigorous sampling. All collected fish were swimming erratically at the water surface and featured prominent hemorrhages along the lateral body surface. On necropsy most of the fish had minimal fat stores and apparently were not feeding. The gills appeared blanched with congestion and hyperemia of select internal organs. Retroperitoneal hemorrhages were apparent in a few fish. Blood was collected for cytologic evaluation and determination of packed cell volume (PCV). At time of collection, blood from specimens appeared bright red and thin, suggesting anemia. PCV's from four fish ranged from 15 to 22 %.

Fresh and formalin-fixed samples were submitted for analysis at the Animal Health Center, Abbotsford, for virology, bacteriology and histopathology. All samples were negative by the polymerase chain reaction procedure (PCR) and cell culture for infectious hematopoietic necrosis (IHN), infectious salmon anemia (ISA), as well as *Piscirickettsia salmonis* and *Aeromonas salmonicida*. However, as previously demonstrated by the DFO, samples were positive for the North American variant of VHS. In

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Pilchard (*Sardinops sagax*) die-off along B.C. coast (Continued…)

addition to VHS, histopathology also revealed that the fish were infected with a fungus, *Ichthyophonus* sp and a chlamydial-like agent, *Epitheliocystis* sp; both of these organisms are found sporadically in wild fish and are considered potential pathogens for salmonids. Microscopic examination of stained blood smears were unremarkable; there were no intra-erythrocytic inclusions compatible with viral erythrocytic necrosis (VEN) which is known to occur within phylogenetically related and intermingled, wild herring populations. Other incidental findings included the presence of nematodes and trematodes in 2 of 5 submitted samples.

Historically, similar die-offs within the pilchard population in Queen Charlotte Strait and other areas along the Pacific Coast have been documented. Although the British Columbia coast was littered with canneries processing pilchards as part of the commercial fishery in the early 1900's, over -fishing is believed to have led to a decline in numbers. Today, pilchards are seen in B.C. during the summer months, but in winter they usually migrate south to California. Despite speculation, reasons for the fish remaining in northern waters this past winter are unknown. Local environmental conditions are implicated in this most recent die-off.

The significance of VHS and other infectious conditions in relation to the mortality among these pilchard stocks is difficult to determine, since dying and moribund fish lacked significant, attendant pathologic lesions. Underlying environmental factors cannot be ruled out as contributing to the losses. Farmers have altered their management strategies to reduce stress on the fish (less handling/grading), and are closely monitoring stocks for increased morbidity or mortality or for fish exhibiting unusual signs of disease.

Formalin and its safe use

J. Coates

Formaldehyde (HCHO) is a powerfully disinfectant organic gas, while the term formalin usually refers to formaldehyde gas in stock 40% aqueous solution. The chemical is regularly used within this laboratory in the preparation of fixed tissues from fresh specimens that originate from necropsies done at our laboratory, or that are sent in from practitioners. We also regularly receive samples for examination and histopathology that have been previously preserved in 10% formalin solution at veterinary clinics.

Occasionally, submitted samples that we receive by bus or courier reek of formalin vapor due to leakage of container bottles or plastic bags. Sometimes, when doing necropsies, we unintentionally spill formalin solution over the necropsy area, or onto the floor. Sometimes this clear, watery, deceptively harmless-looking fluid contacts our hands, and, rarely, our eyes. It is an irritating, foul-smelling, obnoxious, albeit essential chemical for the fixed preservation of fresh tissues.

In clinical practice, there are equally potential hazards with respect to skin contact with formalin solution and inhalation of its vapors that are just as real and worrisome as those that staff at the AHC must contend with. These range from the sloshing of formalin solution within jars or canisters within practice vehicles; spillage in small clinic- laboratory areas during the mixing of formalin solution and dispensing it into shipment-size jars; and chronic exposure to low-level formalin vapors in poorly ventilated storage or "back-room" laboratory areas.

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Health hazards of formalin: the occupational health hazards of formaldehyde and formalin solution are due to its toxic effects after inhalation, after direct contact with the skin or eyes by formaldehyde in liquid or vapor form, and after ingestion.

Inhalation: formaldehyde is highly irritating to the upper airways. The concentration of formaldehyde that is immediately dangerous to life and health is 100 ppm; concentrations above 50 ppm can cause severe pulmonary reactions in minutes. Formaldehyde can cause symptoms of bronchial asthma in humans. The mechanism may be either sensitization of the individual by exposure to formaldehyde or direct irritation in persons with pre-existing asthma. Upper airway irritation is the most common complaint and can occur in concentrations over 1 ppm. Symptoms of dry throat, itching and burning sensations of the nose may occur, or nasal congestion. Tolerance to this level may develop within 1-2 hours. This tolerance can permit workers remaining in an environment of gradually increasing formaldehyde concentrations to be unaware of their increasingly hazardous exposure.

Eye contact: concentrations from 0.05 ppm and 0.5 ppm produce sensations of irritation, with itching, burning, tearing. Tolerance may occur. Accidental splash injuries of human eyes to aqueous solutions of formaldehyde (formalin) can result in a wide variety of injuries, including corneal opacity and blindness. Severity of reactions are directly dependent on the concentration of formaldehyde in solution, and the amount of time lapsed before medical intervention.

Skin contact*:* chronic exposure to formaldehyde solutions can lead to an allergic contact dermatitis. Exposure to solutions or vapors, even when airborne concentrations are well below 1 ppm., can produce these skin reactions of erythema, edema, and hives.

Ingestion: we won't even discuss this.

Chronic effects of exposure*:* long term exposure to formaldehyde has been shown to be associated with an increased risk of cancer of the nose and accessory sinuses, nasopharyngeal and oropharyngeal cancer, and lung cancer in humans. Animal experiments provide conclusive evidence of a causal relationship between nasal cancer in rats and formaldehyde exposure. Formaldehyde is a complete carcinogen and appears to exert an effect on at least two stages of the carcinogenic process.

In British Columbia, the Workman's Compensation Board has set permissible, maximum exposure limits over an 8 hour period at 0.3 ppm, with a maximum tolerated ceiling at any one time of 1.0 ppm.

Safe handling of formaldehyde in your veterinary hospital: Avoid skin contact with formalin solution of any concentration. Avoid inhaling its fumes. With chronic exposure, you will lose your ability to smell or detect the chemical at low concentrations. Unless your facilities are equipped to permit you to handle this material safely (e.g., effective fume hoods that actively vent the vapors form a working site), you should minimize your contact or exposure to the substance as much as possible.

Wear gloves when handling the solution. When diluting solutions or adding to bottles of fresh tissue, add cold water rather than warm, to discourage fume formation. Have your fume hood checked regularly to determine if it is functioning properly; just because the motor is making a sound does not mean it is exhausting fumes effectively. If you pour formalin solution into a sink, wash it down immediately with fresh water, and avoid the fumes; ideally, the sink should be covered by a fume hood and

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Formalin and its safe use (Continued…)

be actively vented. If you store this chemical, or store preserved specimens, do so in a properly vented shelf or in an area that is naturally well vented, and preferably as far away as possible from your working area. Don't take this stuff for granted – look what it does to the tissue that you preserve in it – it's your health that is on the line, and its effects can be insidious.

Shipping of tissues in formalin solution: When you send us your fixed tissues for examination, be sure the package is carefully sealed to avoid leakage en route. If tissues can be fixed before sending them to us, remove most of the formalin solution in the jars or plastic whirlpacks and enclose a few pieces of formalin-soaked gauze or towel. This will ensure their preservation and safe delivery, and avoid the inherent risk of leakage from formalin-filled containers.

Are your employees aware of the hazards of formaldehyde ? Check out the safe handling of formaldehyde in your veterinary hospital today, not only for your own protection, but for staff members that work with you in the same environment. If you are the employer, your responsibility is more than merely ethical or thoughtful. And lastly, give this small article to your employees, and encourage each of them to read it.

– detailed data and statements describing effects of formaldehyde taken from bulletins and regulations of the Occupational Safety and Health Administration, U.S. Dept. of Labor, June/99.

Grey Owl and parasitism:

"Grey Owl's lasting appeal seems a combination of his remarkable story and the truth of his words - his concern for forests and wildlife that find resonance in the modern environmental movement, and instructions for proper behavior toward animals. "Every living thing is parasitical to some degree," he wrote in one of the scolding essays in *Tales of an Empty Cabin,* "but man extracts tribute from everything, even including the less fortunate of his own kind. Almost always he extorts far beyond his needs, destroying without thought for the future - the parasite supreme of all the earth. And in spite of the high position he has gained, he has still much to learn of tolerance, moderation and forbearance towards not only the lesser of created things, but towards his fellow man."

— from the article *The Vision of Grey Owl*, by Dane Larken; *Canadian Geographic*, March/April 1999, vol 119: p 74.

A famous (wooden) horse:

"…Equo ne credite, O Teucri. Quidquid id est, timeo Danaos et dona ferentes…."

(English translation from the Latin: "Don't trust the horse, O Trojans. Whatever it is, I fear the Greeks, especially bearing gifts…").

– cautionary words spoken by Laocoon, a Trojan priest of Neptune, warning his countrymen not to wheel the Wooden Horse into the city's gates. He was ignored; the Horse was wheeled in, and on that fateful night, Troy fell. The Greek gods, Zeus in particular, were angry at Laocoon for having the audacity to warn the Trojans of their impending doom, since the gods had decided, after much squabbling, that Troy would fall to Agamemnon and his Greek compatriots. Helen would be returned to her homeland.

As punishment for attempting to warn the Trojans, Laocoon and his two sons were dragged into the sea by two great serpents sent by Neptune, and drowned.

The story is recorded in Book II of the Aeneid, by the Roman poet Virgil (70 B.C.- 19 B.C.).