Wyoming State Veterinary Laboratory Newsletter – January 2009

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MESSAGE FROM THE DIRECTOR

This past year, the American Association of Veterinary Laboratory Diagnosticians' (AAVLD) past president and past director of the WSVL, Dr. Donal O'Toole, was responsible for a man-power survey of veterinary diagnosticians. Dr. O'Toole has summarized the results for this study. I think you will find these results interesting if not somewhat alarming.

Don Montgomery
Director, WSVL

A diagnostic opportunity for medical microbiology students and veterinarians?

A recent survey by the American Veterinary Medical Association noted that the number of new veterinary graduates pursuing post-DVM qualifications has increased every year since 1995. Currently 36.8% of recent veterinary graduates in the US pursue advanced qualifications. At the same time, state diagnostic laboratories throughout the US and Canada have noticed that it is becoming increasingly difficult to find and hire trained diagnosticians. To resolve what is going on, the American Association of Veterinary Laboratory Diagnosticians (AAVLD) commissioned a survey of state veterinary diagnostic laboratories to get baseline data on the veterinary diagnostic job market. Survey questions were drafted by veterinary diagnosticians in Wyoming, California, Washington state and Ontario. Results of the survey, which involved responses from >60% of laboratory directors in the US and Canada, were presented by Dr. Donal O'Toole at the annual meeting of the AAVLD this October. The main findings of the survey were:

- In recent job searches, the main shortages reported were in pathology, administration and bacteriology among diagnosticians, and in bacteriology, histotechnology, molecular biology and virology among technicians.
- The most important reasons for failed searches for personnel were absence of adequately trained personnel, and compensation package.
- One third of all state laboratories hired diagnosticians without preferred qualifications in the past five years.
- There is a mismatch between what laboratories are training individuals in (at present, overwhelmingly in anatomical pathology) and the disciplines that are needed (especially in diagnostic microbiology, particularly personnel comfortable with both classical and molecular methods).
- The single most important reason for vacancies is that diagnosticians are being hired by other diagnostic laboratories.

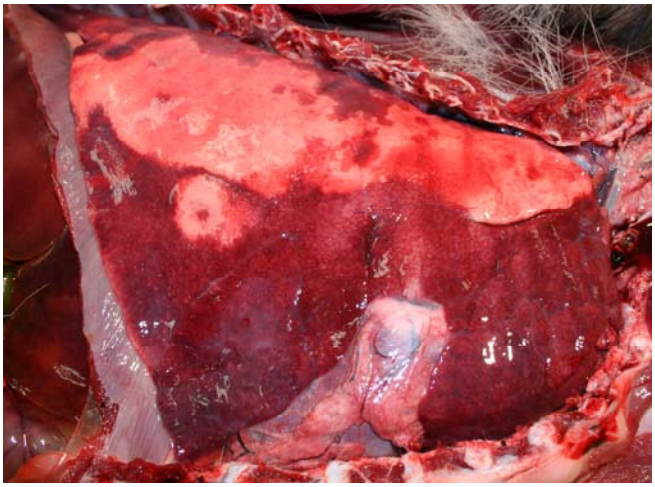
There is no national training program for veterinary diagnosticians or diagnostic technicians. This is in contrast with the situation on the human medicine side, where diagnostic career paths are well defined through state public health laboratories and the Centers for Disease Control and Prevention. As a university that a) runs a state veterinary diagnostic laboratory b) prepares pre-veterinary students for professional programs and c) trains students in applied (classical and molecular) microbiology, the University of Wyoming is in a position to meet these needs. In the absence of a national program, which is unlikely in the present financial climate, it will boil down to state universities, possibly in consortia, to meet these needs.

Donal O'Toole
Pathologist, WSVL
Past President, AAVLD

INTERESTING CASES FROM WSVL AND OTHER TIDBITS

Pasteurella multocida and RSV pneumonia and otitis in a Bighorn Sheep

A female bighorn lamb was presented to the WSVL after being found dead by the Nebraska Game and Parks Commission in early December. Most sheep in the group were reported to be coughing. As we commonly see in bighorn lambs, there were lesions typical of acute bronchopneumonia.



Anterior-ventral bronchopneumonia in a bighorn sheep.

Pasteurella multocida was isolated from this lesion. In addition, bovine respiratory syncytial virus was identified by fluorescent antibody testing on pieces of lung. Histologically, some bronchiolar epithelial cells contained eosinophilic cytoplasmic inclusions consistent with the finding of BRSV by FA testing. Currently, we don't know if Bighorn sheep have their own strain of respiratory syncytial virus that cross-reacts with the BRSV antibody, or whether true bovine RSV is circulating in the population. Jackie Cavender, from the virology laboratory, is currently attempting to isolate the virus. The lamb also had numerous ear ticks within its external ear canal, identified as *Otobius megnini* by Katie Bardsley in the parasitology laboratory. In one ear there was a

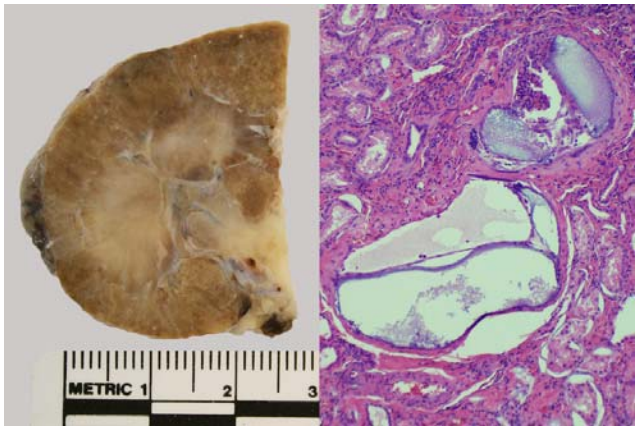
purulent otititis that was confirmed histologically to involve the middle and inner ears.

Jonathan Fox
Pathologist, WSVL

Rottweiler juvenile-onset renal failure with distinctive glomerular changes

A veterinarian in Powell submitted fixed kidneys from an 8-month old Rottweiler with renal failure. The bitch had polydipsia from the time the owners obtained her as a pup. No other signs of clinical disease had been noticed. The bitch was spayed uneventfully at 7 months of age. Terminally the bitch began vomiting.

At necropsy the veterinarian noted both kidneys were irregularly pitted and firm. Grossly they were 50 x 30 x 20 mm and 25-g (left) and 55 x 35 x 30 mm and 52-g (right). They were firm with radial areas of pallor in the cortex and medulla and were gritty on sectioning. White 1 mm foci were throughout the cortex.



Kidney from young Rottweiler bitch with renal failure. Grossly (left), kidneys was fibrotic and gritty. Histologically (right), renal corpuscles were large due to marked dilation of Bowman's space, accompanied by varying degrees of atrophy of glomeruli, so called cystic glomerular atrophy.

Histologically there was chronic pyelonephritis. In addition, there were severe and distinctive glomerular changes consistent with cystic glomerular atrophy. Glomeruli were varied from small to essentially absent, and exhibited partial mineralization and adhesions to Bowman's capsule. The striking feature was the degree of dilation of Bowman's space, which also contained a variable amount of mineral. Basement membranes, including that of Bowman's capsule, were mineralized.

There have been two reports of juvenile-onset nephropathy in Rottweilers, some of which were littermates. A distinctive feature was cystic glomerular atrophy. Ultrastructural examination of affected kidneys from two affected Rottweilers revealed distinctive changes in glomerular basement membrane. Similar "basket weave" splitting of thickened lamina densa is seen in Alport syndrome in people. Alport syndrome disease affects type IV collagen in glomerular basement membrane (GBM), leading to renal failure and death in the absence of dialysis or renal transplantation. Cystic glomerular atrophy is seen in the human disease and the role of abnormal collagen in the juvenile nephropathy of Rottweilers remains to be established. Since this is a fairly popular breed in Wyoming, I'd be interested to know whether any of you has ever seen what you suspect to be a familial form of renal failure in Rottweilers.

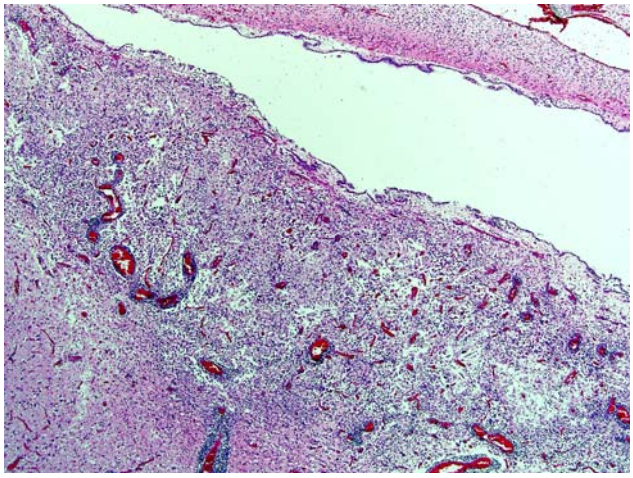
Donal O'Toole
Pathologist, WSVL

Cook et al: 1993, J Am Vet Med Assoc 202: 107 – 109.
Wakamatsu et al: 2007, Vet Pathol 44: 96 – 100.

Probable Case of CAEV Neurological Disease in a Dwarf Goat

We received the carcass of a nine month-old, male castrate, Nigerian dwarf goat with a clinical history of acute onset of neurological signs at initial presentation at three months of age. The primary clinical signs included blindness, anorexia, and abnormal behavior including secluding himself from the remainder of the flock. The kid was treated medically for

enterotoxemia, listeriosis, and polioencephalomalacia and provided extensive nursing care at home where he improved slightly, although vision never appeared to return to normal and some neurological deficits remained static. The goat re-presented approximately five months after the initial bout of illness with head pressing, circling, excessive salivation, and was treated again with medical and supportive care, but failed to improve. At necropsy the goat was emaciated with serous atrophy of remaining adipose tissue and had fecal staining of the tail, perineum, and hind legs with concomitant watery to mucoid and minimally hemorrhagic enteric contents. The brain and entire spinal cord were removed intact and samples of brainstem/cerebellum and spinal cord were submitted to the rabies, bacteriology, virology, and toxicology laboratories for associated testing with the remainder of the tissue fixed intact in 10% NBF. When the brain was sectioned for histopathology, extensive necrosis, malacia/cavitation, and mineralization were observed bilaterally in white matter tracts throughout the cerebrum, especially in periventricular areas. Microscopically there was a severe, subacute to chronic, disseminated and locally extensive nonsuppurative encephalitis with leukoencephalomalacia and mineralization, again most severe in periventricular white matter tracts of the cerebrum.



Severe inflammation in periventricular brain areas in a dwarf goat with suspected CAEV infection.

No gross or microscopic lesions were observed in numerous sections of spinal cord. Additionally the goat had a mild to moderate enteritis with villous blunting, superficial villous necrosis and mild hemorrhage, and abundant intralesional coccidia (*Eimeria* sp.).

Most of the common causes of neurological disease in goats were ruled out through histopathology and laboratory testing (including rabies, listeriosis, and polioencephalomalacia) and the clinical history on this accession together with the gross and microscopic lesions in the goat's brain were strongly suggestive of neurological disease caused by infection with caprine arthritis and encephalitis virus (CAEV). Infection with this lentivirus usually occurs via ingestion of colostrum or milk and transmission most often occurs from infected dams to offspring, but transmission also may occur through prolonged intimate contact between infected hosts and naïve animals and iatrogenic transmission has been described (using the same needle on multiple animals). If neurological disease occurs, it is most common in kids between two and four months of age and neurological signs usually have an abrupt onset and may progress rapidly to death or remain static without significant recovery. Most often in goats clinical signs reflect motor dysfunction in the spinal cord, without evidence of cerebral involvement, but occasionally lesions and resultant clinical signs may be caused by cerebral-predominant or cerebral-only lesions, as in this case. Other common clinical syndromes caused by CAEV infection in adult goats include chronic progressive polyarthritis and interstitial mastitis. Confirmation of CAEV infection can be achieved using a variety of tests including PCR, virus isolation, and immunohistochemistry. Serology is useful for screening herds for infection, but is not particularly useful for confirming CAEV as the cause of clinical signs in an individual animal for a variety of reasons (long seroconversion time, false negatives, and

poor correlation between seropositive status and active disease).

Current recommendations for dealing with this disease in goat herds include removal of kids from seropositive dams at birth, feeding heat-treated colostrum and pasteurized milk until weaning, serological testing and segregation or culling of seropositive goats, and only using seronegative goats from CAEV-negative herds as replacement animals. Keep in mind that CAEV is a reportable disease in sheep and goats in Wyoming, and suspect or confirmed cases should be reported to the state veterinarian's office within 24 hours.

1.http://www.cfsph.iastate.edu/Factsheets/pdfs/caprine_arthritis_encephalitis.pdf (accessed 01/12/2009).

2. Ravazzolo, AP, C Nenci, HR Vogt, et al. (2006). Viral load, organ distribution, histopathological lesions, and cytokine mRNA expression in goats infected with a molecular clone of the caprine arthritis encephalitis virus. Virology, 350:116-127.

3. Rowe, JD, and NE East (1997). Risk factors for transmission and methods for control of caprine arthritis-encephalitis virus infection. Veterinary Clinics of North America Food Animal Practice, 13:35-53.

4. Storset, AK, O Evensen, and E Rimstad (1997). Immunohistochemical identification of caprine arthritis-encephalitis virus in paraffin-embedded specimens from naturally infected goats. Veterinary Pathology, 34:180-188.

Todd Cornish
Pathologist, WSVL

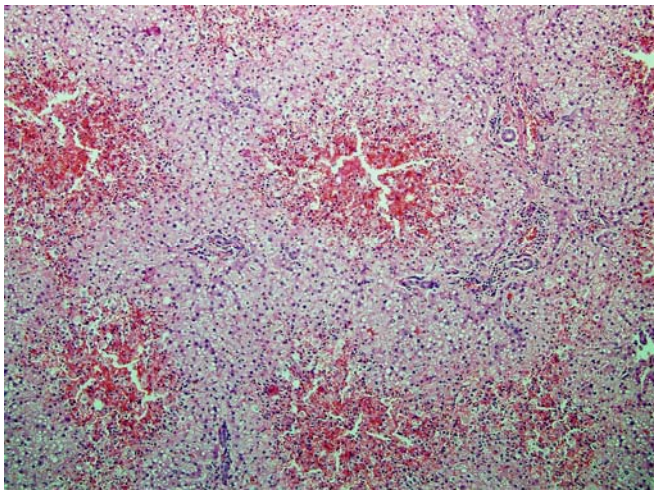
Cocklebur Poisoning in Cattle

Multiple samples from three 10 month-old stocker calves were submitted to the WSVL. The calves were being fed a ration composed of millet hay, wet beet pulp, and whole corn and had access to protein blocks containing Rumensin (monensin). The calves all displayed a variety of neurological signs antemortem, including behavioral changes (apprehension, aggression), staggering, muscle twitching/fasciculation, recumbency, and opisthotonos and a total of seven calves were affected within a 12-hour period. No gross lesions were observed in a heifer calf, but in a steer calf the referring veterinarian described a variety of mild subcutaneous and internal hemorrhages, pulmonary congestion, and a diffusely mottled liver. Given the neurological signs observed differential diagnoses included polioencephalomalacia, thromboembolic meningoencephalitis (*Histophilus somnus*), metabolic diseases (especially hypomagnesemia or grass tetany), and various forms of toxicosis including lead poisoning. Several calves were treated with some combination of broad-spectrum antibiotics, thiamine, and/or calcium gluconate with no response. While tissues from two animals were processed for histopathology and laboratory testing, a complete blood count and serum chemistry panel were run on one heifer, and significant findings included marked elevations in ALP, AST, GGT, CK, and LDH activities and a moderate leukocytosis with neutrophilia. These findings were consistent with acute hepatic injury, possibly with some degree of cholestasis, concurrent muscle damage, and a nonspecific inflammatory response.

Laboratory testing on these accessions essentially was unrewarding (no significant bacterial isolates, no viruses isolated or detected to date, parasitology findings unremarkable, etc.). Microscopically in sections of liver from two calves there was diffuse, acute, and marked hepatocellular necrosis/apoptosis in centrilobular (periacinar) and midzonal areas, consistent with a toxic hepatopathy.

Consultation with the referring veterinarian revealed that the hay component of the ration contained a significant amount of common cocklebur (*Xanthium strumarium*) fruits or burs, and this finding together with the hepatic lesions and clinical pathology findings supported a diagnosis of cocklebur poisoning in the affected animals. In subsequent analytical testing, carboxatractyloside (see below) was detected in rumen content. No new clinical cases have been

observed since the millet hay was removed from the ration early in the outbreak.



Centrilobular hepatocellular necrosis with sinusoidal congestion in a calf exposed to cocklebur in hay.

Common cocklebur is a coarse herbaceous annual, common throughout much of the United States (including most of Wyoming), and grows to a mature size of 2-5', with an erect, often angled stem, and alternate, triangular or heart-shaped rough leaves. The plant produces hard, prickly, oval fruits or burs approximately ¾" long containing two seeds – these can be found entangled in the coats of livestock and long-haired dogs not infrequently. The plants are invasive and often are found growing in pastures and meadows (especially those with a history of previous or seasonal flooding), along fencerows, in roadside ditches, along stream and pond banks and in dried out ponds or stock reservoirs, and occasionally in disturbed areas in feedlots. Cocklebur poisoning is most common in spring or early summer, associated with the ingestion of germinated seeds and palatable young dicotyledon seedling plants that are high in the toxic principle – carboxyatractyloside (a sulfated glycoside) – adult plants contain relatively little toxin, other than in seeds or burs. Most poisonings seem to occur in pigs foraging naturally, but the plant is toxic to a wide range of animals, including ruminants, horses, dogs, rats, and humans (there are several reports of fatal poisoning in humans associated with ingestion of cocklebur) and most cases of toxicity in these species are caused by incorporation of seedlings or mature plants with seeds into feed rations (hay, haylage, silage, or grain rations). As with this case, there are several reports of poisoning in cattle associated with the presence of mature cocklebur plants and seeds (burs) in hay. Common clinical signs observed with cocklebur poisoning include anorexia, depression or other behavioral changes including apprehension or excitability, blindness, ataxia, twitching progressing to spasmodic muscle contractions or convulsions, recumbency, opisthotonos, and rapid progression to death. Clinical signs may follow ingestion of the plant by as short a period as several hours in monogastric animals and may be delayed for a day or so in ruminants. Characteristic gross lesions of cocklebur poisoning are not specific, but can include ascites and various effusions, hepatic swelling, congestion, and mottling, fibrin tags on serosal surfaces of viscera, renal congestion, and gastrointestinal congestion. Microscopic lesions generally are confined to the liver, with characteristic centrilobular (periacinar) to midzonal hepatocellular degeneration, necrosis, and apoptosis with congestion/hemorrhage, although lesions also may be observed in the kidney and brain on occasion. Diagnosis of cocklebur poisoning generally requires some combination of: 1) evidence of ingestion of cotyledonary seedlings or seeds/burs, 2) appropriate history and clinical signs, 3) characteristic clinical pathology findings, and 4) consistent gross and microscopic lesions. Diagnostic assays that detect the toxic principle in tissues or other biological samples have been or are being developed, but none are routinely or widely available to veterinary diagnosticians.

Treatment of affected animals generally is unrewarding once clinical signs have progressed to the neurological stage, and there is no antidote for the toxic principle, but supportive care and therapy aimed at increasing GI clearance of

ingested plants, decreasing GI absorbance of the toxin, and treating metabolic and neuromuscular complications all have been shown to be effective on occasion. Prevention of poisoning is more effective than treatment of clinical cases, and can be achieved by elimination of plant populations (mowing before seed production begins, use of herbicides, limiting access to contaminated pastures and meadows, manual removal of plants from hay fields, etc.).

1. Martin, T, EL Stair, and L Dawson (1986). Cocklebur poisoning in cattle. Journal of the American Veterinary Medical Association, 189:562-563.
2. Mendez, MC, RC dos Santos, and F Riet-Correa (1998). Intoxication by *Xanthium cavanillesii* in cattle and sheep in southern Brazil. Veterinary and Human Toxicology, 40:144-147.
3. Stuart, BP, RJ Cole, and HS Gosser (1981). Cocklebur (*Xanthium strumarium*, L. var. *strumarium*) intoxication in swine: review and redefinition of the toxic principle. Veterinary Pathology, 18:368-383.
4. Turgut, M, CC Alhan, M Gurgoze, et al., (2005). Carboxyatractyloside poisoning in humans. Annals of Tropical Paediatrics: International Child Health, 25:125-134.
5. Whitson, TD, et al., (2001). Common cocklebur, *In* Weeds of the West 9th Edition, TD Whitson (ed.), Western Society of Weed Science/Western United States Land Grant Universities Cooperative Extension Service/University of Wyoming, pp 194-195.
6. Witte, ST, GD Osweiler, HM Stahr, and G Mobley (1990). Cocklebur toxicosis in cattle associated with the consumption of mature *Xanthium strumarium*. Journal of Veterinary Diagnostic Investigation, 2:263-267.

Todd Cornish
Pathologist, WSVL

Johne's PCR

Two years ago we began to offer a PCR for Johne's disease as a rapid alternative to culture, which can take several months to complete. We have gotten very few requests for the PCR and the reagents are quite expensive so we will no longer offer the test. Reagents go out of date after 6 months and it is $1,000 to order in a fresh set. We will return to sending out Johne's culture to Wisconsin. WSVL will continue to offer the ELISA for serological diagnosis of the disease.

Ken Mills
Bacteriology Section, WSVL

FROM THE WYOMING DEPARTMENT OF HEALTH

Reporting of Human Illness in Wyoming

The Wyoming Department of Health (WDH) has established two toll-free numbers that can be utilized by the public to report human illness outbreaks, potential outbreaks, and individual cases of reportable diseases in humans. The phone numbers are **877-996-9000** for reporting during the daytime and **888-996-9104** for reporting during non-business hours. The phone numbers can also be utilized to arrange for consultation with subject matter experts on infectious disease and other public health issues. Veterinarians can refer individuals to these numbers when faced with the uncomfortable and relatively common situation of being asked to diagnose and treat a client's illness. WDH encourages veterinarians to also use the phone numbers to report knowledge of any confirmed or suspected reportable disease in humans. See http://wdh.state.wy.us/phsd/epiid/reporting.html for a list of reportable disease and conditions in humans in Wyoming.

Karl Musgrave, DVM, MPH
Wyoming Department of Health