Wyoming State Veterinary Laboratory Newsletter – June, 2007

University of Wyoming
Department of Veterinary Sciences
1174 Snowy Range Road
Laramie
WY 82070
http://wyovet.uwyo.edu/

Main office/Director
Phone: 307 742 6638
800 442 8331 (Toll-free Wyoming only)
Fax: 307 721 2051

To phone laboratories directly
307 742 6681 + EXT. BELOW

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Dr. Gerry Andrews	766 3139
Dean Frank Galey	766 4133

WNV Information
call Dr. Todd Cornish
307-742- 6681 Ext. 191

http://uwadmnweb.uwyo.edu/VETSCI/

MESSAGE FROM THE ACTING DIRECTOR

The staff of the WSVL hope this issue of the Newsletter finds you well and having an enjoyable early summer. Some of you may be interested in some personnel additions and changes that are anticipated for the Laboratory.

Last year, the Veterinary Sciences Department and WSVL received approval to add a position for a fourth anatomic veterinary pathologist. A search, begun early in 2006 and extending into January of 2007 did not result in a successful hiring. A new search is currently being conducted and, although the applicant list is short, we are excited about the slate of candidates that have expressed an interest in this position. This brings up another important issue concerning the future of diagnostic laboratories that are supported by public funding. For several years now, it has become increasingly difficult to attract veterinarians with advanced training in non-clinical disciplines to careers in academia and diagnostic veterinary medicine. There are many reasons behind this. Many new veterinary graduates, already burdened by educational debt, can see no immediate economic advantage from three up to five or even six years of additional specialized training. Many of those who do are attracted to the higher salaries and modern state-of-the-art facilities offered by industrialized veterinary medicine. Lastly, funded training programs in the diagnostic specialties have not kept pace with an ever increasing demand for these services. Where this will lead us is difficult to predict.

Another note regarding personnel issues, Donal O'Toole who has served tirelessly as Director of WSVL and Head of the Veterinary Sciences Department has expressed an interest in stepping down at the end of his five-year term in September to return to full-time service as a pathologist, to teaching, and to pursue research interests. A replacement has not officially been announced at this time. His skill as an administrator and national advocate for diagnostic veterinary medicine will be missed but, on a lighter side, all will benefit from his knowledge and many years of experience in diagnostic pathology. I hope you will join the personnel of WSVL in expressing your gratitude.

Donald (Don) L. Montgomery
June 14, 2007

DIAGNOSTIC CASES OF INTEREST

A Breachy Bull

Recently, a veterinary client of the Vet Lab described an event in which a *Tritrichomonas foetus*-infected bull breached a fence and entered a pasture inhabited by 150 young cows that were near the end of synchronization in preparation for artificial insemination. The bull was seen with the cows the day after the fence was breached, and was immediately removed. Contact time was thought to be approximately 24 hours or less.

Pertinent questions regarding this situation included:

- Should the cows be tested for infection before AI?
- How many of the cows were likely to have been bred and thereby exposed to the protozoan during the time the bull was in the herd?
- How long will the infection persist in the infected cows?
- Are any or all infected cows likely to abort or reabsorb fetuses after AI?
- How can we determine whether cows that are open at the end of the pasture season were unproductive due to the protozoan parasite?

Possible (not totally definitive) answers to the questions:

- Culture and/or PCR analysis of 150 vaginal samples would cost between $1,050 and $4,000, for lab testing alone. The cost of sampling and shipping of samples would add to that figure. Many cows would have sparse vaginal infections, which would complicate finding the protozoa in the mucosal scrapings. The time and expense involved, and the low probability of finding the organisms in the relatively few infected animal(s), may negate sampling and testing benefits.
- The number of cows bred by the bull in the time he was in the herd is hard to estimate; much would depend on the enthusiasm and energy level of the bull and the receptive attitude of the cows.
- The vaginal infections are thought to persist through one or two estrous cycles, with spontaneous clearance in MOST (not all) cows within 90-100 days of sexual rest. A very few cows maintain a chronic infection, and may even produce calves.
- It is highly likely that any infected cows in this herd, due to be inseminated within days of contact with the infected bull, would lose a fetus to the ravaging protozoan.
- In this individual case, a single, old bull is scheduled to be turned out with the cows after AI, to breed any of the females that happened to require clean-up insemination during pasturage. If any of those infected, cycling, open cows are contacted by the 'clean-up bull,' he will likely acquire an infection from contact with the cow. Testing a prepucial scraping of that bull at the end of the season would provide a somewhat-confident indication as to whether one or more of the cows were infected by the "breachy-bull."

In a more common situation, in which all cows in a herd are bred by bulls during pasturage rather than inseminated by AI, one or more of the herd bulls would be likely to acquire a *T. foetus* infection from any cow(s) infected by a breachy bull. The newly infected bull(s) would then transmit the agent to a number of other cycling cows in the herd. The end result would be a significant drop in calf production, and the need to test, cull and send infected bulls to slaughter.

Sampling and testing cows for *T. foetus* can be done, and has identified some infected cows in Wyoming on at least 2 occasions since 1985, but is less efficient and much more expensive than testing bulls. The expense involves the relative number of cows, compared to the number of bulls in a herd that need to be tested. If there is evidence that indicates the presence of a chronically infected but productive cow in a herd, testing of the cows may be the only way to identify and cull the reservoir animal. The estimated prevalence of chronically infected cows capable of producing healthy calves is less than 1 percent. Since the majority of infected cows have been shown to spontaneously clear a trichomonad infection within 90 to 100 days of sexual rest, most of them can be cycled back into the herd for the next breeding season, or culled as soon as they are found open.

Bill Jolley
May 31, 2007

Neurological EHV-1

Equine herpesvirus type 1 (EHV-1) is an important horse pathogen responsible for several diseases. Respiratory signs such as nasal discharge and cough are the most prominent but the virus may also cause abortions, perinatal death and neurological disorders. Acute forms of the disease are mainly seen in young animals while older animals are usually asymptomatic. Like other alpha herpesviruses, EHV-1 can remain latent for the horse's life after primary infection. Reactivation can occur as a result of any stress condition, leading to the shedding of virus and infection of other animals. Neurological signs may or may not accompany the respiratory syndrome and they vary from mild incoordination to asymmetric ataxia. Sometimes the animals exhibit difficulty urinating or defecating. Since infection occurs very early and is for life, vaccines are only be useful to reduce the period of viral shedding during reactivation and to limit reinfections.

Recent publications suggest that the virus strains associated with neurological disorders have a distinctive characteristic in the sequence of one gene (polymerase gene). The polymerase gene is a non structural gene which does not induce specific antibodies during infection. For this reason a serologic diagnosis of this strain is not possible, nor can current vaccines protect against this strain.

The only way to determine whether the infecting virus carries this particular polymerase is to perform PCR with later sequencing or through a real time PCR. PCR is a technique

based in the amplification of a conserved region of the EHV-1 genome for its later visualization in a gel

Diagnosis of a "neuropathogenic strain of EHV-1" however, will not change the prognosis of the disease or later treatments although stress factors which can reactivate this particular strain, may be avoided.

The WSVL offers EHV-1 viral isolation and EHV-1 PCRs for diagnosis of the agent. The laboratory has also developed the PCR/sequencing test which specifically detects the neuropathogenic strain. The WSVL have recently applied this approach to determine the genomic characteristics of two commercial attenuated EHV-1 live vaccines. The results have indicated that both vaccines have been developed with strains carrying the genomic marker characteristic of neuropathogenic strains.

Nicki Bratanich
6/1/2007

Zoonotic potential of Salmonella

Within the last couple of months, I have heard some misinformation concerning the potential transmission of Salmonella to both animals and humans. A quote from a recent Pan American Health Organization book ***Zoonoses*** states "**With the exception of *S. typhi* and the paratyphoid serotypes (particularly A and C), which are species-specific for man, all other infections caused by Salmonella may be considered zoonoses. Salmonella is perhaps the most widespread zoonosis in the world.**" Certainly there are differences in the infectivity of various Salmonella serotypes but none, except those mentioned above, should be considered non infectious for other animal species or humans.

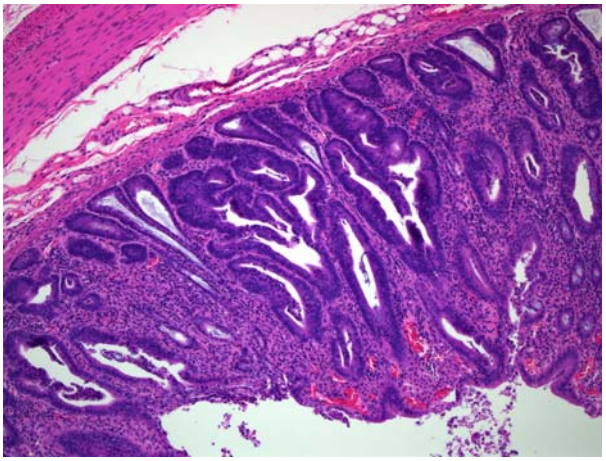
Since we are on the subject of Salmonella I would like to just mention a few interesting facts.

- These bacteria resist dehydration for an extremely long time in fecal material as well as food.
- They can survive for several months in a brine of 20% salinity.
- A British study demonstrated that they can survive up to 14 months in facilities occupied by infected calves.
- Equine clinics that have infected animals are very difficult to clean as evidenced by the experience of a number of Veterinary teaching hospitals over the past few years.

Ken Mills
6/3/2007

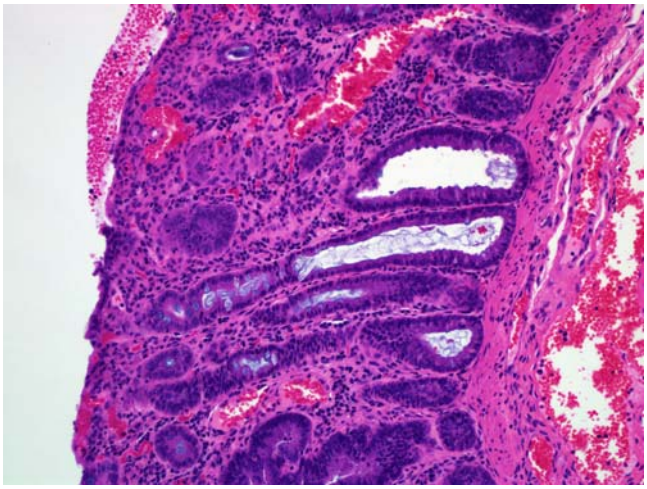
Swine Dysentery

A 3-month-old pig developed bloody scours and died. Select fresh and fixed tissues were submitted to the Wyoming State Veterinary Laboratory. Proliferative enteritis with crypt abscesses and superficial necrotizing colitis were seen on microscopic examination of the small and large intestines. The first lesion is compatible with proliferative ileitis and the second is most compatible with swine dysentery.



Proliferative ileitis *(Lawsonia*) – note marked tortuous hyperplasia of crypts.

Proliferative ileitis is caused by *Lawsonia intracellularis* (previously *Campylobacter*-like species). Infection with this organism can cause a very proliferative lesion (porcine intestinal adenomatosis), a proliferative bloody enteritis with a luminal blood clot (proliferative hemorrhagic enteropathy) or a necrotizing lesion (necrotic enteritis). Cases are typically seen in post-weaned fattening pigs between 6 and 20 weeks of age although proliferative hemorrhagic enteropathy can



Swine dysentery – note colonic glands are distended with mucus and superficial epithelium is necrotic and attenuated (*Brachyspira hyodysentery*)

also be seen in young adults. Diagnosis is typically made using histopathology and demonstration of organisms within enterocytes by special stains. Immunohistochemistry and PCR is also available.

A targeted medication program is recommended following infection on a farm. Tylosin in the feed and lincomycin-spectinomycinin in water is the recommended regime which should reduce losses from infection without compromising

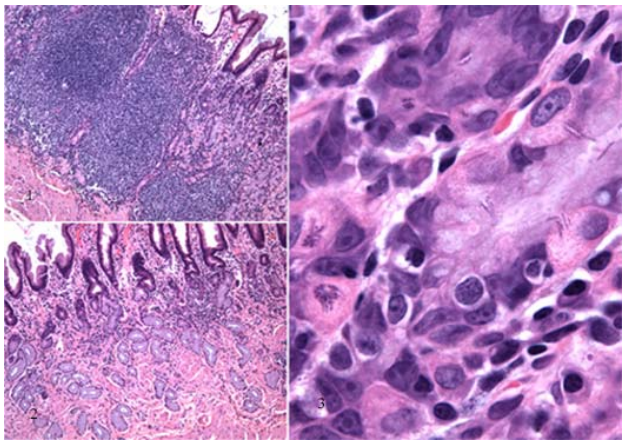
development of immunity. Chlortetracycline commenced after exposure has also been shown to be effective. One vaccine has been shown to be efficacious and more vaccines are in development.

Swine dysentery is caused by *Brachyspira hyodysenteriae* (previously *Treponema* and *Serpulina*. This disease primarily affected pigs during the growing-finishing period (15-70 kg pigs) but can also occur in adults, particularly sows reared outdoors and occasionally in suckling pigs.

Leslie Woods
6/15/2007

Helicobacter-associated gastritis in the dog

Bacteria having spiral or helical morphology have been recognized in the stomachs of humans and animals for many years. The discovery of *Helicobacter pylori* in humans and its association with a variety of syndromes including acute and chronic lymphofollicular or atrophic gastritis, gastroduodenal ulcer, and gastric cancer, has led to heightened interest in Helicobacter species in animals. Several *Helicobacter* species are currently recognized in animals but their pathogenicity varies. The relevance of these organisms to gastritis in dogs is not universally accepted.



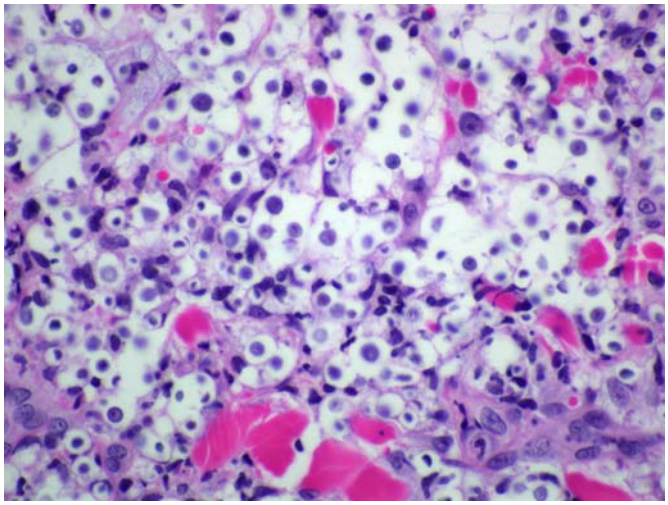
Lymphofollicular gastritis (1), gastric mucosal atrophy with loss of parietal and chief cells (2), and Helicobacter-like bacteria (3).

This spring WSVL received gastric biopsies from an 11-year-old Walker hound who had demonstrated a painful abdomen after eating. Gastric biopsies were taken revealing a severe lymphoproliferative gastritis progressing to atrophy in some areas (see figure). Further examination revealed numerous bacteria having helical morphology on the luminal surface and, more importantly, extending deep into the mucosa. These organisms were present in areas with, but not without inflammation. Lymphofollicular gastritis may also be observed in dogs in the absence of *Helicobacter*-like organisms. In a past similar case, a veterinary gastro-enterologist recommended treating a dog with identical lesions for these organisms and, if clinical signs persisted, trying to alleviate the inflammation by appropriate immunosuppressive therapy. In humans, triple therapy for *Helicobacter pylori* is recommended. Therapy consists of a gastric protectant, proton pump inhibitors such as pantoprazol, and dual antibiotic therapy. Antibiotics that have been used include amoxicillin, clarithromycin,, metronidazole, and others. At least a 2-week period of treatment has proven more efficacious than one week.

Don Montgomery
6/15/2007

Cryptococcosis in a dog

A 2 year old Husky-cross was diagnosed with cutaneous cryptococcosis. *Cyptococcus neoformans* is the etiologic agent of which two variants and five serotypes have been identified. The organism is ubiquitous in nitrogen-rich, alkaline debris and soil contaminated with pigeon excrement. Infection with the organism is seen more frequently in humid environments. Cutaneous cryptococcosis is very rare in dogs and typically accompanies systemic infection. Infection has been associated with immunosuppressive diseases such as neoplastic disease or diabetes mellitus, and with iatrogenic immunosuppression. Cases in dogs preferentially affect the central nervous system and the eyes. Rare cases of cutaneous crytococcosis in dogs reported disseminated ulcers and **gure**



Cutaneous cryptococcosis

fistulas affecting the muzzle, oral cavity, lips and clawbeds. Lesions in this dog were seen on the ears. The American Cocker Spaniel is reported to be predisposed in North America and the Doberman Pinscher and Great Dane are at greater risk in Australia. This dog was a Husky cross. In one report of cutaneous cryptococcosis in humans, treatment included systemic administration of amphotericin B (AMB), 5-flucytosine and triazole agents such as fluconazole and itraconazole combined with topical ketoconazole cream.
Leslie Woods 6/15/2007

OTHER ITEMS OF INTEREST

Wyoming—Part of the Woodtick World

Veterinarians, ranchers and other people in and around our brushy, beautiful, animal-friendly state have noticed an impressively larger-than-usual population of ticks in Wyoming this year. Many calls to the Parasitology lab in the WSVL refer to the large numbers of ticks attached to cattle, horses and dogs. Common questions asked by the callers include concerns about where the bugs are coming from, why are they so numerous this year, what are the dangers of being bitten, how can they be dealt with (controlled) and when will they go away?

Most people know that when the ticks need a blood meal, they crawl up on vegetation, usually sagebrush and other low shrubs in areas inhabited by animals. When a warm-blooded animal touches the plant on which the ticks are perched, the hungry little creatures merely step onto the animal and crawl to a preferred area on the animal's body before feeding. The animal hosts may be rodents, rabbits/hares, coyotes and other carnivores/omnivores, and herbivores of all kinds, including deer, elk, moose, antelope, cattle, sheep and horses. Humans are included in the carnivore/omnivore list.

This year's impressive population indicates that egg production was significant during the last year or two, winter survival of eggs was high and probably most importantly, the rodents, rabbits, hares and/or other small animals fed on by the larvae and nymphs of the 3-host ticks have had high production and survival. Some adults have a lifespan of two or more years and if climatic factors (many of which are not well known) are favorable, their reproduction increases with their longevity. If the larval and nymphal stages are blessed with easy access to large numbers of blood meals, more ticks will reach adulthood, resulting in higher egg production for the next year. 2007 appears to be one of those years favorable to our native 3-host ticks. Our common 3-host ticks include *Dermacentor andersoni* (Rocky Mountain Wood Tick) and *Dermacentor viriabilis* (Kennel Tick/American Dog Tick). *Rhipicephalus sanguineus* (Brown Dog Tick) may feed on 3 different hosts if necessary but more commonly has access to, and preference for, canines on which any/all of the stages are often found.

One-host ticks normally crawl onto a herbivore host in the fall season, and stay on that host throughout the winter, feeding and molting from late fall throughout the winter to early spring, during which time the larval, nymphal and adult stages develop without having to leave the host. They drop off the host only after the female tick has been fertilized and needs to lay eggs. Two 1-host ticks we see commonly in Wyoming are *Dermacentor albipictus* (Moose Tick/Winter Tick) and a soft tick species, *Otobius megnini* (Spinose Ear Tick).

Effects of the bite of our most common Wyoming tick, *D. andersoni* (Rocky Mountain wood tick), may range from nothing more than localized swelling, itching and burning, to anemia, tick paralysis, Rocky Mountain spotted fever/Tick fever, Colorado tick fever, Query/Q fever, Tularemia and Anaplasmosis. Not all of these conditions appear in people or animals parasitized by our ticks in Wyoming every year, but some of our native tick species are capable of causing the conditions or transmitting the infectious agents that cause the diseases. In areas endemic for the infectious agents that cause the RM spotted fever (a rickettsia), Colorado tick fever (a virus), Q fever (a bacterium), Tularemia (a bacterium) and Anaplasmosis (a rickettsia), surveys have consistently found low percentages of the tick populations to carry the disease-causing agents.

Anemia is simply blood loss caused by large numbers of the nymphs and/or adult ticks engorging on blood before dropping off the host to complete the next life cycle stage in their normal development. Tick paralysis results from neurotoxins injected into a host during a blood meal; as the tick feeds on a host, chemicals including the neurotoxins are injected into the bite site to promote blood flow into the digestive organs of the parasite, preventing clotting that will plug the bug. Susceptibility of animals (and humans) to tick paralysis depends on concentration levels of the toxins in the tick saliva and on species, age and other host-related factors.

Dogs are especially susceptible to RM spotted fever, and should be considered indicators of risk to their owners. The infection is quite common in Wyoming and other areas where *D. andersoni* and some related ticks are common. The rickettsiae can be acquired by the larval, nymphal or adult ticks, retained by the life cycle stages through molting or egg development, and passed on to a subsequent host fed on by any of the feeding stages. Typical early clinical signs include fever, anorexia, vomiting, diarrhea, facial edema, coughing, ocular petechial hemorrhages and other visible effects.

Colorado tick fever results from a Coltivirus for which *D. andersoni* is a primary vector. Symptoms may include fever, headache, muscle pain and stiffness, joint aches, eye pain, light intolerance, chills, sore throat, nausea and others. The virus may develop in many internal organs, including bone marrow and/or neural tissue. Colorado tick fever as the name suggests, is found in Colorado more commonly than in other western states.

Tularemia caused by *Francisella tularensis* causes headache, nausea, fever, enlarged lymph nodes, pneumonia and an ulceration at the bite site. Many species of ticks transmit the agent to numerous wild and domestic animals, humans and birds. It is most common in wild rodents, rabbits and hares. *Coxiella burnetii*, the bacterium that causes Q fever, is most common in ruminants, but other domestic and wild animals have also been infected with it. Clinical signs in humans are wide-ranging, from self-limiting flu-like, to endocarditis, hepatitis and/or pneumonia-like. It is highly infectious, and can be acquired via aerosol inhalation.

The one-host ticks are most likely to cause anemia, because of the long period of time they infest a single host animal. The larval stages that feed first are the smallest stage, the nymphs significantly larger than the larvae and the adults larger and more voracious than the earlier stages. In addition to blood loss, alopecia is a common sign of infection, especially on moose and elk that have been infested with significant numbers of *D. albipictus* for several months. The spinose ear tick *Otobius megnini*, is rather common in pronghorn antelope, deer, bighorn sheep, cattle, domestic sheep, goats, horses, dogs, humans and other animals. It can transmit RM spotted fever, Q fever, Tularemia and Colorado tick fever, but the most common damage is severe irritation of the ear canal and promotion of secondary infection with screwworms and bacteria. It can also cause tick paralysis.

Control is sometimes difficult, but in Wyoming is almost always focused on direct treatment of animals that harbor significant tick populations. Acaricide treatment of vegetation in large pasture/range areas common in our state is impractical. In domestic animal housing it may be useful, but in most cases domestic animal infections direct application of acaricides by spraying, dipping, implanting, bolus administration, collar/ear tag application and vaccines are less expensive and more effective than treatment of plants. Organochlorines, organophosphates, carbamates, amidines, pyrethroids and avermectins have been used for the longest periods of time. Recently, several new compounds have been developed to overcome resistance some of the ticks have developed to the older chemicals. The phenylpyrazoles are some of the newest anti-tick drugs now on the market.

The ticks listed and superficially discussed here are the most common that we are aware of in Wyoming. Other species have been shipped to WSVL for identification, but in most cases those not considered native have been transported into our state on companion, competition or food animals. The reports we've gotten from Wyoming veterinarians about the large numbers of ticks on their clients' animals predominantly involve our common 3-host *Dermacentor* species, that have benefited from peak production of rodents and lagomorphs that have generously provided a rich source of blood to the larval and nymphal tick stages.

Bill Jolley
6/15/2007

Effects of Chronic Wasting Disease on Survival and Mortality of White-tailed Deer in Southeast Wyoming

Chronic wasting disease (CWD) is a transmissible spongiform encephalopathy, or prion disease, of mule deer (*Odocoileus hemionus*), white-tailed deer (*Odocoileus virginianus*), Rocky Mountain elk (*Cervus elaphus nelsoni*), and moose (*Alces alces*). Other prion diseases include bovine spongiform encephalopathy, or mad cow disease, in cattle, scrapie in sheep, and Creutzfeldt-Jakob disease in humans. Exactly how CWD is transmitted between animals, how it is spread geographically, and how it impacts affected free-ranging deer and elk populations are poorly understood.

We are performing a study in southeast Wyoming to determine effects of CWD on behavior and survival of white-tailed deer and provide information on spread of CWD. Deer were captured as fawns, tested for CWD, marked with ear tag radio-transmitters, and recaptured on a yearly basis to re-test for CWD and replace radio-transmitters with global positioning system collars. In five years, we have captured, marked and CWD tested 154 deer. The overall CWD prevalence in the study population is 27% (37/136).

David Edmunds performing a tonsil biopsy of an anesthetized study deer.

To determine how CWD is affecting the deer population, we calculated the percent of deer that were alive at the end of each year (annual survival). Based on this analysis, survival rates are lowest for adult CWD-positive deer (0.29; female = 0.22; male = 0.40). Annual survival rates are significantly lower for adult CWD-positive deer than CWD-negative deer and adult female CWD-positive deer than female CWD-negative deer. Our findings suggest CWD is lowering annual survival of white-tailed deer and may limit deer population growth.

David Edmunds and Todd Cornish
6/15/2007

BVDV Detection/testing Update

Between the fall of 2006 and spring of 2007 a small number (<10 samples) of ear notches in PBS or formalin AND blood from the same animal(s) were received at the WSVL for BVDV testing. Samples were submitted from cattle operations in WY and surrounding states. Tests requested were BVDV antigen capture ELISA (AgELISA, PBS ear notch test, ACE, produced and distributed as IDEXX Herdchek® by IDEXX LABORATORIES, Westbrook, ME)

for ear notches in PBS and serum, or, in the case of formalin-fixed ear notches, IHC for BVDV. In all cases, the AgELISA detected BVDV in the ear notches but failed to detect BVDV in some of the blood samples. This scenario held true for formalin-fixed ear notches tested by IHC and serum (or plasma) from the same animal(s) using the AgELISA. All animals sampled had clinical signs suggestive of BVDV infection and/or mucosal disease.

The ACE was originally developed and validated for use with ONLY serum (ear notches in PBS were validated for use in the AgELISA several years after serum) so this unusual finding caught our attention immediately. To ascertain whether this was a repeatable change in the detection rate of BVDV in blood with the IDEXX Herdchek® ELISA, ten PI calves were held at the WSVL for 3 months to sample and compare ear notch detection rates with that of blood. Detection rates in the AgELISA were validated by IHC in the case of ear notches and virus isolation in the case of blood. We found that the antigen-capture ELISA detected BVDV in 100% of the ear notch samples, as did IHC, but detected BVDV in only 66% of the serum samples collected concurrently. Virus isolations on lymphocyte preparations detected BVDV in 100% of these blood samples.

Because of this finding, we are recommending that only ear notches (minimum 1cm^2) in 2 mls of PBS be submitted for BVDV testing with the antigen-capture ELISA. Blood may still be used for BVDV detection by virus isolation and PCR. We are presently conducting research to determine why BVDV is becoming difficult to detect in blood products.

Please call Dr. Nicky Bratanich 307-742-6681 ext. 161 or Jackie Cavender 307-742-6681 ext. 162 in the virology laboratory with any questions.

From: Wyoming State Veterinary Laboratory
Department of Veterinary Sciences
University of Wyoming
1174 Snowy Range Road
Laramie, WY 82070
http://wyovet.uwyo.edu/