What are we up to?

_Veterinary Medicine Extension_ is still working on the Bovine Respiratory Disease Risk Reduction Project. We participated in the _WSU Beef Production Conference_ in Yakima June 13th to talk about vaccinations. About 200 people were at the conference learning about beef marketing, genetics, etc.

The _Field Disease Investigation Unit_ conducted a number of investigations with students and assisted producers and veterinarians with phone and email requests for information. Investigations and information requests included: cattle vaccinations, colostrum information, PEDV, pig mortality, copper and selenium deficiencies, low pregnancy rates in beef cows, calf loss, cattle respiratory disease and weight loss in dairy heifers.

The _Population Health Research_ group just submitted a couple of USDA grants, one looking at alternatives to antibiotics and mitigating antibiotic resistance and the second as part of a national dairy cow lameness project.
Kasimanickam R, Kasimanickam V, Kastelic JP. Mucin 1 and cytokines mRNA in endometrium of dairy cows with postpartum uterine disease or repeat breeding. Theriogenology. 2014 Apr 15;81(7):952-958. Mucin (MUC) 1 is an inducible innate immune effector, an important component of defense against bacterial invasion, and is linked with infertility in humans. The objectives were to evaluate messenger RNA (mRNA) expression of MUC1 and cytokine genes in the endometrium of cows with various postpartum uterine inflammatory conditions or with a history of repeat breeding. Endometrial samples were collected from lactating dairy cows diagnosed with metritis (n = 4), endometritis (n = 4), subclinical endometritis (n = 4), or no uterine pathology (normal; n = 4). In addition, endometrial samples were collected from repeat breeder cows with (n = 4) or without (n = 4) subclinical endometritis, and unaffected cows (n = 4). Quantitative polymerase chain reaction was used to determine mRNA abundances of MUC1, Toll-like receptor (TLR) 4, interleukin (IL) 1β, IL6, IL8, tumor necrosis factor (TNF) α, insulin-like growth factor (IGF) 1, and IGF-binding protein (BP) 2. The mRNA expressions were significantly greater for cows with metritis and clinical endometritis compared with cows with no uterine inflammation, except for IL6. However, mRNA expressions for these target genes were not different for cows with subclinical endometritis, compared with cows without uterine inflammation, except for IL1β and TNFα mRNA (P < 0.01). All mRNA expressions were greater (P < 0.001) for repeat breeder cows with subclinical endometritis compared with normal cows. However, in repeat breeder cows without subclinical endometritis, only expressions of MUC1, IGF1, and IGF BP2 were greater compared with normal cows (P < 0.01). Based on functional protein networks, there were significant associations between these transcripts. In conclusion, endometrial expressions of MUC1 and cytokine genes differed among normal, fertile versus diseased, and subfertile dairy cows. Perhaps, these altered gene expressions contribute to endometrial insufficiency and consequently pregnancy wastage.

Park KT, Allen AJ, Barrington GM, Davis WC. Deletion of relA abrogates the capacity of Mycobacterium avium paratuberculosis to establish an infection in calves. Front Cell Infect Microbiol. 2014 May 15;4:64. Previous comparative studies in goats revealed deletion of relA but not pknG abrogates the capacity of Mycobacterium avium subsp. paratuberculosis (Map) to establish a persistent infection. The immune response elicited by the mutant cleared infection. The objective of the present study was to extend the studies in calves and compare the proliferative response elicited by the relA deletion mutant (ΔrelA) and Map using flow cytometry and quantitative reverse transcription real-time PCR (qRT-PCR). Six 3-day-old calves were divided into two groups. Three were vaccinated with ΔrelA and 3 inoculated with wild type Map. The calves were challenged with Map 1 month later and necropsied 3 months post challenge. Three untreated calves were used as uninfected controls. Examination of tissues revealed the ΔrelA mutant was immune eliminated. Bacterial load of Map was significantly reduced in the calves vaccinated with ΔrelA and challenged with Map in comparison with calves inoculated and challenged with Map. A vigorous CD4 memory T cell response was detected at necropsy in PBMC from both infected groups. CD8 positive NK cells proliferated in the presence and absence of antigen stimulation in both treated groups but not in the uninfected group. IFN-γ, IL17, and IL22 gene expression were up-regulated with an associated increase in their transcription factors, Tbet and RORC, in both treated groups. TGF-β, IL-10, and FoxP3 were not up-regulated, indicating no activation of regulatory T cells. The findings show that the immune response to ΔrelA is clearly different than the response to Map. Understanding the immunological basis for this difference should facilitate development of a vaccine that elicits sterile immunity.

[From the Editor: Johnes disease control with vaccines has been fraught with difficulties. If a vaccine could be developed that reduced total bacterial load and shedding, herd control of this chronic disease would be much easier. Right now, we are left with biosecurity and specific control programs using testing and culling to reduce infections in herds. For more information See: http://www.johnes.org/dairy/control.html]
What’s New at WADDL?

Cause for Concern: Bovine Leukemia Virus Update
By Dr. James Evermann

Where have we been?
Bovine leukemia (leukemia) virus infection has been monitored in the United States cattle populations since the late 1970’s when a serologic assay was first introduced. Prior to that time there was sporadic clinical evidence dating back to the late 1800’s that cattle were susceptible to infectious cancer. The agent was first identified in 1969, and that discovery allowed for development of various diagnostic tests. Over the past 40 years testing has allowed for us to monitor for BLV infection from its subclinical phase to the clinical phase, and construct reliable control strategies for BLV infection in herds, regions, and in some cases, complete eradication.

Initially, there were two reasons to control BLV. They were first centered on reduction of carcass condemnation at meat processing plants, and the second was to improve trade-marketing of cattle within regions and between countries. Since overt clinical forms of bovine leukemia were being noticed less due to cows shorter duration of time on the farm (primarily dairy cattle), trade restrictions between countries were the predominant reasons to test for and certify populations of cattle as “BLV free”.

Recently, there has been renewed interest in controlling BLV within the United States, not only for improvement of trade-marketing of cattle, but also because of newer data, which affirms that BLV infection has a negative effect on dairy cattle production. These data, in addition to reports of BLV genomic segments being found in human tissues have prompted this update.

Bovine Leukosis - Why test for it?
The question really should be, why not test for it? The test using serum has very good sensitivity (98%) and specificity (100%) and, although not licensed by USDA, the test for milk has good sensitivity (95%) and specificity (99%). In repeated studies over the past ten years, it has been demonstrated that BLV infection is not just subclinical, but that there are demonstrable clinical effects and production losses associated with infection. Several recent reports indicate that BLV infection reduces milk production, increases death losses, and has a negative effect on the dairy industry in general. Bartlett et al. reported that BLV-infected animals were more likely to be culled early from the herd or die. This evidence raises awareness that there is a range of disease manifestations associated with BLV infection in addition to lymphosarcoma. What we used to call...
subclinical infection is now seen to manifest slowly and is more analogous to a chronic debilitating type of BLV syndrome.\textsuperscript{3}

While effects of BLV infection on the animal are important, there is increasing laboratory data that demonstrates BLV proviral (viral DNA) sequences within human tissues.\textsuperscript{5,10} This observation has been speculative before, but with increasing test sensitivity using molecular diagnostics, the data are more convincing. Authors of these reports indicated that further analysis is required before a direct cause and effect can be determined. However, in the best interest of the dairy industry it would be prudent to move towards a voluntary BLV eradication program over the next few years, the recommendation from Drs. Janice Miller and Martin Van Der Maaten over 30 years ago.\textsuperscript{15}

**What options do we have for controlling BLV infection?**
There are at least four options to consider when talking about BLV control (Table 1).\textsuperscript{3} The first is that **no actions are taken** to test for or remove BLV test positive cattle, the primary approach taken in the US. The second would be where the **herd is monitored for BLV infection by blood or milk based antibody testing**, and that **management changes** (Table 2) are instituted in an effort to reduce spread of the virus. This practice has been instituted on many farms throughout the country and can be effective in reducing herd prevalence within several years. The third and fourth options are similar, in that initially **all cattle are tested**, the **herd is maintained as closed**, and only BLV test negative cattle are added to the herd. The major difference is that in option three, animals are segregated depending upon their BLV infection status. In essence, maintaining two subpopulations, one BLV test negative, and one BLV test positive. This option has benefits of a “phase BLV infection out process”, which then leads to option four, which is characterized by culling any BLV test positive cattle and using strict biosecurity on all incoming cattle.

**Table 1. Summary of options for BLV control in United States dairies**\textsuperscript{*}

1. No action taken
2. Monitor for BLV infection by testing for BLV specific antibodies in serum or milk. Make comprehensive or selected management changes (Table 2) to reduce spread of BLV.
3. Test all cattle and separate out BLV test positive cattle. Make selected management changes to reduce spread of BLV. Maintain a closed herd or only add BLV test negative cattle (two negative tests 30 days apart).
4. Test all cattle and cull BLV test positive cattle. Maintain a closed herd or only add BLV test negative cattle (two negative tests 30 days apart). *modified from Bartlett et al, 2014\textsuperscript{3}*

\*modified from Bartlett et al, 2014
What management changes can decrease BLV spread within and between herds?

Management changes that result in decreasing BLV infection in a herd are multiple and largely animal age dependent. Herds studied in the Northwest showed a stair-step like increase in BLV infection over the lives of the animals (Fig 1). A number of infections occurred in utero, but was quite variable (3-20%), due perhaps to the animal’s genetics or the percent of cattle with lymphocytosis during their pregnancy, or both.

The second peak of infection occurred during calfhood, in which up to 40% of infections took place. This may have been due to feeding colostrum from BLV positive cattle, spread of infection by blood contaminated instruments (dehorners, ear tagging pliers, ear tattooers, etc.), or by common-use needles during vaccinations, injectable treatments, etc.

### Table 2. Recommended management changes to decrease BLV spread within dairy herds*

1. Use separate needle for each animal
2. Clean/disinfect blood-contaminated equipment for tattooing, ear tagging, dehorning, extra teat removal, and other surgical procedures between animals
3. Use a new or cleaned rectal palpation sleeve for each cow.
4. Use AI exclusively for breeding purposes
5. Control stable and other biting flies
6. Segregate BLV test positive cattle from BLV test negative cattle
7. Cull BLV test positive cattle with lymphocytosis
8. Minimize contact between newborn calves and BLV test positive cattle.
9. Avoid feeding unpasteurized colostrum from BLV test positive cows to newborn calves.4

*Modified from Bartlett et al, 2014

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**Figure 1.** Schematic demonstrating the three age-related risk periods for bovine leukemia virus (BLV) infection (Cumulative percentages*). Modified from Evermann et al, 1987. *Percentages are independent of herd additions of BLV-infected cattle.
The third peak was noted at the heifer/mature cow ages, and doubled the infection rate in the herd up to 80%. Again, as was noted for calfhood infection, blood contaminated instruments and needles are critical in the spread of the virus. In addition, rectal palpation was considered to be a risk for spread of BLV on some dairies.

**What is the risk of spreading BLV infection in the herd?**

Another way to look at BLV spread is to categorize what particular management procedures are at higher risk compared to others. In Table 3, procedures are divided into thirds: calfhood; reproductive; and housing and confinement. For each category, there is a risk of either high or low assigned to that particular procedure. The common element amongst the high risk procedures is blood, where it has been demonstrated that it only takes a fraction of a drop (0.001 ml) of blood to infect an animal.

**Table 3. Risk of BLV spread in cattle under production conditions**

<table>
<thead>
<tr>
<th>Risk</th>
<th>Calfhood Procedures</th>
<th>Reproductive Management</th>
<th>Housing/Confinement</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>Gouge dehorning with a common instrument</td>
<td>Rectal palpation with a sleeve</td>
<td>Contact with blood, tissues, and fluids at parturition</td>
</tr>
<tr>
<td></td>
<td>Other surgical procedures permitting blood transfer</td>
<td></td>
<td>Contact between cattle in herds with high BLV prevalence</td>
</tr>
<tr>
<td></td>
<td>Intravenous injection or blood draw with a common needle and/or syringe</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>Ear tagging</td>
<td>Natural breeding</td>
<td>Hematophagous insects</td>
</tr>
<tr>
<td></td>
<td>Tattooing</td>
<td>Artificial insemination</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Subcutaneous, intradermal, or intramuscular injections</td>
<td>Embryo transfer</td>
<td></td>
</tr>
</tbody>
</table>


**What can the laboratory do to assist in screening for BLV infection?**

Since the majority of cattle will seroconvert (develop antibodies) far in advance of clinical signs, it is important to test animals at key times in their production cycle. Antibody testing is the most common and inexpensive assay. There are PCR assays available, but they are not validated by the USDA, and are generally used to detect varying loads of virus in circulation or body tissues for research.

Serum-based and milk-based ELISA are available nationally and internationally, and are generally regarded as the OIE gold standards. Depending on the option(s) used to control BLV infection, testing can be done annually on the resident herd, and on all replacement animals (two negative tests 30 days apart).

The WADDL tests for BLV antibodies on serum samples every Tuesday. The turnaround time is usually 24-48 hours. If any questions arise, please contact the Consulting Microbiologist at 509-335-9696, or Dr. Evermann at 509-339-3607. Refer to the WADDL website for copies of the accession and multiple animal ID forms www.vetmed.wsu.edu/depts_waddl
Conclusion
New evidence points to the effects of BLV on cattle health and performance. However, the infection and associated disease can be managed in, and in some cases eradicated from a herd with testing and a variety of management changes.

Acknowledgements - Gratitude to the WSVMa Bovine Leukosis Committee (Drs. V. Pedersen, R. Glore, P. Dahlquist, E. Studer, R. DiGiacomo, S. Hopkins, R. Darlington) who embarked upon a state-wide control, education and testing program. Appreciation to the Immunodiagnostics Section at WADDL for running the BLV assay.

References

Keeping Calf Hutches Cool
By Dr. D.A. Moore

Heat stress in calves affects feed consumption and daily weight gain, particularly if they cannot get cooled at night. In May of this year, researchers at Texas A & M reported on a reflective fabric they used to radiate the heat of the Texas summer sun off of calf hutches. They were able to lower the heat in the hutch at calf lying level by over 8 degrees. They are still working on the details but hope to have a system that will cost about $4.00 per hutch. If these covers are not yet available, what can a dairy farmer do now to reduce hutch heat in summer?
(1) Elevating the hutch - We recently reported on a way to increase ventilation in plastic hutches by raising the back with an 8 X 8 X 16 inch concrete block (http://extension.wsu.edu/vetextension/Documents/CalfHeatStressTrial2012.pdf). This method reduced interior hutch temperatures, reduced carbon dioxide levels, and improved air flow.

(2) Shade cloth - A number of studies going back into the 1980’s demonstrated the effects of shade cloth over calf hutches to reduce respiratory rates, reduce hutch temperature and improve average daily gain. Shade cloth with 80 percent barrier to solar radiation can be used. Some considerations - make sure the shade cloth is high enough over the hutches to prevent stagnant air and higher humidity underneath the shade. The height of about 11 feet above the ground has been recommended. When not in use, the shade cloth can be rolled up and strapped to the support posts.

(3) Hutch Orientation - Another strategy is to maximize the available shade provide by the hutch itself. In the western US, orienting the hutches to the north in the summer will do just that.

(4) Provide plenty of fresh water - Calves will drink more during the summer and if they have a case of scours will need even more water.

For our courses and factsheets on calf housing and environments, see: http://extension.wsu.edu/vetextension/calfscience/Pages/CalfHousingEnvironment.aspx

Porcine Epidemic Diarrhea Vaccine

On June 16th, the USDA announced a conditional license for a Porcine Epidemic Diarrhea (PED) vaccine. The disease PED causes illness and death particularly in young pigs but can affect swine of all ages. It had been found in Europe and Asia for many years and reached the US just a little over a year ago and has spread to many states. USDA recently issued a Federal Order, “Reporting, Herd Monitoring and Management of Novel Swine Enteric Coronavirus Diseases,” effective June 5, 2014, to help them find other infected herds for control purposes (http://www.aphis.usda.gov/animal_health/animal_dis_spec/swine/downloads/secd_monitoring_management_plan.pdf).

The disease caused by the PED virus is similar to TGE (transmissible gastroenteritis) that has been around the US for some time. TGE and PED are both coronaviruses that affect
only pigs. PED is transmitted from pig to pig through ingested fecal material. The first thing a pig owner might see is diarrhea. The disease can be confirmed by a test of the feces or intestinal contents of a dead pig. To best identify the cause, the samples need to be collected and put on ice within 24 hours of the start of diarrhea and submitted to the diagnostic lab. WSU-WADDL will be proficient in the new testing (a PCR test) within the next couple of weeks.

For more information on PED and novel enteric coronaviruses of swine, see: https://www.aasv.org/aasv20website/Resources/Diseases/PorcinEpidemicDiarrhea.php

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**WSDA Corner**  
**By Dr. Paul Kohrs, Acting State Veterinarian**

We received notice on March 26th that 2 bulls and 31 cows from a herd in S. Oregon with trichomoniasis had entered Washington and ended up close to Vader, WA without a Certificate of Veterinary Inspection and the proper testing. Dr. Gilliom visited the ranch and they are now quarantined until the bulls have 2 negative tests. In April a family emergency in Arizona necessitated the owners deciding to cull the bulls, but only one could be secured and it was followed to sale and slaughter. The remaining bull and cows remain under quarantine with the cows.

On March 14th Dr. Dobbs followed up on another Strangles case that passed through a Sunnyside horse rescue lot on 2/20/14. This particular horse ended up in Oregon, and came down with Strangles a few days later after arriving in Oregon. “Ginger” was treated by a veterinarian, and then she went to a horse boarding facility.

Several Animal Services staff members were involved with and deployed to the Oso SR530 Mudslide. The Washington State Emergency Operations Center sent a mission on April 3, 2014 to activate WSDA’s Reserve Veterinary Corps (RVC). The newly hired RVC coordinator, Dr. Minden Buswell accepted the mission and spent long hours working with responders and RVC members to accomplish the mission. The mission requested that the department provide two veterinarians, two veterinary technicians, and two veterinary vehicles to support the federal and county search and rescue canines that were deployed to the mudslide response. The concerns on the site were contamination from household chemicals, hypothermia, hypoglycemia and traumatic injuries from hazards in the mud. Our veterinary profession should be very proud of all the doctors, technicians and support staff that assisted in the Oso disaster. The mission ended on April 23rd.

In January of 2013 a dairy herd in Moses Lake had a cow that tested positive to Tuberculosis. A mandatory re-test of the 1036 head dairy occurred on April 6th and April 10th. The re-test was comprised of USDA and WSDA veterinarians, including Drs. Dobbs, Gilliom, Itle, and Smith. The re-test resulted in 19 responders that were sent in for additional testing; all came back negative. This completes the Moses Lake Tuberculosis investigation.
Dr. Amber Itle investigated a possible Foreign Animal Disease (FAD) on April 24th and 25th involving a 7+ year old Hereford beef cow that showed central nervous system signs and had a lesion on her muzzle. After getting a full history of the cow, it was determined the risk of FAD is extremely low. The cow recovered, although she remains intractable so no further testing has been done.

Dr. Lyndon Badcoe has continued to monitor the flock with Very Virulent Infectious Bursal Disease (vvIBD). Dr. Badcoe wrote a plan, discussed the objectives with Dr. Kohrs and requested USDA funding to support the composting of manure to destroy vvIBDV. On the animal side of the quarantine, ten days after the placement of specific pathogen free (SPF) poultry in two infected barns, chickens were found dead with typical lesions of vvIBDV. A calendar has been developed to track the composting process. More SPF poultry will be used as sentinels to test for presence of the virus after the barns have been cleaned and disinfected. So far there is no evidence of spread beyond the two barns on the one premise.

The Animal Disease Traceability project is on track and under budget. The Request for Proposal (RFP) for the Livestock Inspection software received responses from two vendors and their capabilities were demonstrated on May 14th and 15th. The department is in the final stages of choosing a vendor to develop this integral piece of the project.

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**Continuing Education**

**Veterinarians**

**Academy of Dairy Veterinary Consultants**

Fall 2014 Meeting will be in October. Location, agenda and date to be announced. To get on the mailing list, contact Dale Moore at damoore@vetmed.wsu.edu

**Producers**

**Bovine Respiratory Disease (BRD) On-Ranch Risk Assessment**

Washington State University Extension has developed an on-ranch risk assessment for cow-calf herds. If you are interested in having a Beef Extension Team member visit your ranch and get one-on-one education about BRD risks, contact Sandy Poisson at: spoisson@vetmed.wsu.edu

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