



## Wisconsin Veterinary Diagnostic Laboratory

UNIVERSITY OF WISCONSIN-MADISON

# Newsletter - Fall 2019

## Message from the Director



Greetings from the WVDL, we hope your fall is going well! Thank you for taking the time to read our quarterly newsletter. We are excited to have some interesting articles and announcements from our Madison and Barron Laboratories.

As you may already know, we no longer have an option to render equine carcasses, making alkaline digestion for necropsied horses our only disposal option at this time. Considering the expense (\$1.50 per pound) of alkaline digestion due to the huge amount of labor and energy that is required to run the digester, we are doing our best to find alternative disposal options.

We are sad to announce that we are closing the Chemistry Section at WVDL, effective November 1st, 2019. Chemistry and toxicology diagnostic testing, for the the most part, have moved to one of 4-5 regional veterinary diagnostic laboratories that have invested heavily in the equipment and expertise to provide exceptional service. WVDL's caseload has slowly declined due to the enormous cost of equipment replacement and we have found referral to be in our (and our client's) best interest. We will, however, continue colostrum and liquid feed testing as we currently provide.

Aside from the articles below, I would like to highlight the changes to policy for forensic and legal necropsy cases including body retention policy. As of November 1st, 2019, we will require a legal entity (Police Department, Animal Welfare Officer, District Attorney) to be associated with all forensic and legal necropsies submitted to WVDL. We are also limiting our body retention to 6 months. You will see changes to the submission form for these cases this month. Please contact us with questions about these important cases.

We have several of our staff planning continuing education and traveling to different events and meetings. See below and our advertisement in the WVMA Newsletter for the upcoming Veterinary Forensics and Autopsy Wet Lab on November 3rd. Please stop by and say hello if you see any of our team at an event and feel free to contact us at any time with questions, comments or concerns.

Keith Poulsen  
Director

## Rotavirus B in Cattle

by Ryan Breuer and Don Sockett

### • To Test or Not to Test

One of the most common causes of bovine neonatal diarrhea is Rotavirus, which may occur around 5 days to 2 weeks of age. Extent and site of destruction to the epithelial cells comprising the microvilli in the small intestine depends on the age of the calf during the time of infection. Early protection is usually gained through adequate colostrum consumption allowing for local protection at the level of the small intestinal lumen. If adequate passive transfer from a vaccinated dam/source is not obtained in a timely manner (colostrum-deprived calves) disease can be seen as early as 24 hours. Over time, acquired immunity will build and neutralizing antibodies will increase with age and exposure. Transmission primarily occurs through the fecal-oral route and clinical signs can be seen 1 to 3 days post infection, with a 5 to 9 day duration. Due to dam immunosuppression at time of partition, there is a heightened risk of infection to the calf as shedding is increased in cows that are carriers of Rotavirus. These carrier cows as well as a contaminated environment present an increased infection risk to their newborn calves. Rotavirus is fairly stable in the environment, and seen to survive in soil and water at less than 41°F for months to years.

There are 7 different antigenically distinct serotypes for Rotavirus (A-F), with Rotavirus A through C being isolated from cattle with serotype A being the most common among calves with diarrhea. Rotavirus B is less prevalent in the cattle population and is more common in lambs. The significance of Rotavirus B is not observed often. Adult cattle are more likely to develop clinical disease from Rotavirus C than non-adults.

With information kindly shared from the University of Minnesota Veterinary Diagnostic Laboratory it was found that Rotavirus B is of lower prevalence (1.2% over the past 10 years), and less significance to cause clinical disease in the cattle population. Please be informed that the Wisconsin Veterinary Diagnostic Laboratory will not be conducting regular standard testing for Rotavirus B. If further rule out of Rotavirus B is desired the WVDL can send samples to a referral lab for additional Rotavirus B testing. The WVDL will maintain PCR testing for Rotavirus A as it is a significant disease to diagnostically test for in cattle.

## Microbiology

### UPDATE: Discontinuation of VagC real-time PCR for the detection of *Salmonella* ser. Dublin

The WVDL works to continuously monitor our assays in order to make improvements and confirm that our assays are sensitive and specific. In doing so, we found two *Salmonella* enterica subspecies enterica serotype Dublin isolates that were not detected by our VagC PCR primers. Upon whole genome sequencing, we found that approximately a 2,000 base pair segment, containing the VagC gene, was no longer present, which led to the false negative results using the VagC PCR assay. In addition, on July 13, 2018, we reported that the WVDL discovered that VagC can be harbored in *E. coli*, *Enterobacter*, and *Citrobacter*. It is still unknown what the prevalence of this gene in non-*Salmonella* ser. Dublin bacteria is. Therefore, the VagC PCR is no longer specific for *Salmonella* ser. Dublin in which it can cross-react with members of the Enterobacteriaceae family and can give false negative results due to a large deletion discovered in two *Salmonella* ser. Dublin isolates. These two factors have led the WVDL to discontinue the use of the VagC rtPCR as of August 26, 2019. The discontinuation of the VagC PCR will not affect the *Salmonella* species PCR, which will still be run. The WVDL is examining other cost-effective ways to identify *Salmonella* ser. Dublin using molecular methods. The WVDL continues to offer culture for *Salmonella* and complete serogrouping and serotyping. As a reminder, the WVDL automatically cultures and serotypes resulting *Salmonella* isolates when a sample obtains a 35 or lower cycle threshold (CT) on the *Salmonella* species PCR.

Since 2015, the WVDL has been collecting data comparing our PCR and culture rates using the data collected from samples that obtain a 35 or lower CT value on the *Salmonella* species PCR. For 2018, we found that 85.2% of samples with a  $\leq 35$  CT value cultured *Salmonella* of any serotype (Table 1). Additionally, we found that a CT value of  $\leq 30$  had a 94.9% culture rate. For 2017, we found that 80.7% of samples with a 35 or lower CT value cultured *Salmonella* of any serotype (Table 2). Similar results were observed for the years 2014 to 2016 (Table 3). Therefore, we feel confident in using our *Salmonella* species PCR along with or followed by *Salmonella* culture will meet our clients' needs. Previous reports demonstrate much lower culture rates for *Salmonella* ser Dublin (0-25%; Nielsen et al., 2013). We believe that *Salmonella* species PCR followed by culturing CT values lower than 35 produces the most cost effective way of obtaining a positive *Salmonella* culture. The tables below demonstrate our culture rates for *Salmonella* post *Salmonella* species PCR.

Table 1: Culture rate for *Salmonella* species post PCR for 2018.

<i>Salmonella</i> species PCR CT Value	Culture Rate

Table 2: Culture rate for *Salmonella* species post PCR for 2017.

<i>Salmonella</i> species PCR CT Value	Culture Rate
≤ 35	80.7%
≤ 30	93.9%
≤ 25	97.6%

Table 3: Culture rate for *Salmonella* species post PCR for 2014-2016.

<i>Salmonella</i> species PCR CT Value	Culture Rate
≤ 35	77.8%
≤ 30	91.3%
≤ 25	97.9%

Note: Culture rate does not take into account animals that were treated with antibiotics prior to submission. We apologize for any inconvenience this may create for you and your clients. We appreciate your business and continued patronage as we work to improve this test. If you have any questions, please see our website or contact us at any time.

### McMaster's EPG Quantitative Egg Count now offered at Barron WVDL

The Barron Bacteriology lab will begin offering McMaster's EPG quantitative egg count on feces starting November 1st. This can be performed on bovine, caprine, equine, ovine, and camelid fecal sample.

### Improvements to the MAP Real-Time PCR Assay

WVDL has completed the validation of new real-time PCR assay for the detection of *Mycobacterium avium* paratuberculosis (MAP) in accordance with AAVLD guidelines. WVDL has improved the specificity and sensitivity of the assay for all species routinely tested. This multiplex real-time PCR assay uses three targets, which minimizes the possibility of cross-reaction with other *Mycobacterium avium* complex members thereby increasing specificity of this PCR assay for MAP. There will be no change in cost for the improvements to the assay, but there will be a change to the reporting structure. Currently, we report results as either positive, negative or inconclusive based on the cycle threshold (CT) value obtained from the sample. Our validation data has shown that there are a small number of exotic samples (less than .01%) that show PCR signals that are not consistent with the true positive samples. Even with utilization of the additional targets, we are unable to conclusively determine the MAP PCR result for these rare situations. Therefore, we have decided that with those situations we will report the MAP PCR results as undetermined, with the additional recommendation of retesting the animal within the next 6 months. If you have any questions, please feel free to contact the Microbiology Molecular Section.

### Escherichia coli Toxin Real-Time PCR

The WVDL has recently validated a new real-time PCR assay for the detection of the following toxin genes: K99 and F41 fimbriae, heat stable enterotoxin (Sta), intimin and two Shiga toxins (Stx 1 and Stx 2). This real-time PCR replaces the conventional PCR for these toxin genes leading to an increase in sample types allowed and a 3 or more day increase in turn-around time as culture of the *E. coli* is not needed. This assay is validated for tissues, feces and bacterial isolates. The in-state cost for this assay is \$65 and out-of-state cost is \$97.50. The assay is run on Tuesday and Thursday, but samples must be received the day prior to testing.

### Listeria monocytogenes Real-Time PCR

WVDL has completed the validation of a *Listeria monocytogenes* real-time PCR assay in accordance with AAVLD guidelines. The assay is validated for tissue, such as brain, and fecal samples as well as bacterial isolates. The in-state cost for this assay is \$28 and out-of-state cost is \$42. The assay is run on Tuesday and Thursday, but samples must be received the day prior to testing.

### Campylobacter jejuni Real-Time PCR

WVDL has also completed the validation of a *Campylobacter jejuni* real-time PCR assay. This assay was developed in coordination with Food and Drug Administration (FDA) Veterinary Laboratory Investigation and Response Network (Vet-LIRN). This assay is validated for dog feces and bacterial isolates. The in-state cost for this assay is \$28 and out-of-state cost is \$42. The assay is run on Tuesday and Thursday, but the samples must be received the day prior to testing.

The FDA Vet-LIRN is an arm of the FDA that promotes human and animal health by collaborating with veterinary diagnostic laboratories to investigate animal illnesses caused by animal feeds or drugs. For more information: <https://www.fda.gov/animal-veterinary/science-research/veterinary-laboratory-investigation-and-response-network>.

## Staff Spotlight: Doug Dawson, Microbiologist

- I was born in Kansas City, Mo. and raised in Independence, Mo. where I graduated from Truman High School in 1995. I received my B.S. in Biology from Drake University (Des Moines) followed by my M.S. in Water Resource Management (UW-Madison).

2. **What is one thing on your 'Bucket List'?**

- To visit the Hockey Hall of Fame in Toronto.

3. **What book(s) are you currently reading?**

- 'Butter - A Rich History' by Elaine Khosrova
- 'Official Book of Hockey Hall of Fame Legends'

4. **What is the one food that you will never bring yourself to eat?**

- Pickled eggs or pretty much anything pickled.

5. **What is your favorite movie soundtrack?**

- Last of the Mohicans

6. **If you could play the lead role in any movie, which character would you choose?**

- Herb Brooks in 'Miracle' because that man knew hockey and knew how to motivate a team.

7. **What three flavors of ice cream would be on your triple scoop cone?**

- Chocolate Peanut Butter - A childhood favorite;
- Strawberry - Because it reminds me of my Dad and summertime;
- Chocolate Shoppe's Exhausted Parent - Because that is the flavor I came up with to win their contest ... and it's still there!!!!

8. **When you were a child, what or who did you want to be when you grew up?**

- All I ever dreamed about was becoming an astronaut and traveling to the outer limits of the cosmos.

9. **What pets did you have growing up?**

- Closest thing I had to a pet was a farm dog named "Mutt" at my paternal grandfather's farm in Maryville, Mo.

10. **What skill would you like to master?**

- Ice skating so I could keep up with my boys.

11. **What conspiracy theories do you believe in?**

- That problematic and potential FAD cases only show up at 4:27 p.m. on a Friday when we're already short staffed.

12. **Best concert you ever saw?**

- My wife Kristine surprised me for my birthday one year and she took me to see Junior Brown at the Stoughton Opera House.

13. **If you could be any animal in the world, what animal would you be and why?**

- Wandering Albatross - I could soar high and far and see as much of the world as I could.

14. **What is the favorite part of your job?**

- The comraderie. I've been a part of WVDL since January of 2003 and have gotten to meet and work with some amazingly great people. Plus, I've learned a lot along the way with just how important we as a lab are to so many customers.

## Weather Changes Mean Calf Management Changes

by Ryan Breuer and Don Sockett, WVDL

- **Reminder to inform cattle producers to develop a plan now to combat change in cooler ambient temperatures as the seasons change.**

temperature range [60-75°F] where a calf does not need to spend extra energy to stay warm or to cool itself.

Therefore, in cold weather, calves must be managed differently to utilize the consumed calories to keep warm as well as grow and maintain a healthy status. Demand for nutrients increases not only during inclement weather but for illness as well. In particular, nutrient demand is seen as a need for increased protein, energy and fat-soluble vitamins. These nutrient needs are required for tissue maintenance, tissue repair, immune function and to provide the body the ability to keep warm.

Calves are born with approximately 3-4% body fat – NOT much to spare in cold weather. If a calf is not fed enough calories in cold weather, it will use the energy that should be used for weight gain, and use it to keep warm. Standard liquid diets consist of feeding 3 quarts of milk (12.5% total solids) or its equivalent in milk replacer (MR) twice daily to provide an 88-lb calf with approximately 0.5-0.75 lbs/head/day of nutrition for growth. If calves experience an inadequate energy balance that is negative for greater than 3-5 days, starvation will occur. Starvation causes body systems to shut down in order to preserve energy. Since ruminants do not metabolize fat as efficiently as non-ruminants, providing more fat in the diet will not satisfy the nutritional demands of the calf to maintain growth, support immune function and heal tissue damage caused by disease such as pneumonia or diarrhea. Below are options to keep calves healthy and gaining in the cold weather.

- Increase caloric intake when calves are below the thermal neutral zone. There are 3 options available:
  - Feed calves 3 quarts of milk or MR, 3 to 4 times daily instead of twice daily. There should be at least 12 hours between the first and third feedings of the day. This is the best option (especially in extreme cold) because calves will benefit from **more frequent** feeding allowing them more calories distributed over a 24 hour period.
  - Continue feeding the calves twice daily but **increase the volume** of milk or MR per feeding from 3 to 4 quarts.
  - Continue to feed 3 quarts of milk or MR twice daily but **increase total solids** content (12.5-15%).  
CAUTION: To avoid negative issues with too high total solids, producers should work closely with their herd veterinarian and nutritionist if they choose this option.
- Provide fresh warm water shortly after each milk/MR feeding (within 15-30 minutes) to all calves from birth to weaning. The colder the weather, the warmer the water calves will tolerate; aim for body temperature. When calves are laying down remove their water so it will not freeze.
- Provide fresh, clean, highly palatable calf starter/grain that is high in protein (18-22%) daily. When it is cold, calves will consume more to obtain adequate energy for growth and to keep themselves warm. Starter is another excellent way to provide an energy source to calves during cold weather and it can be introduced to calves at 2-3 days of age.
- Bed calves in a draft-free environment with clean, dry straw to nest in – deep enough to cover the calf's legs completely.
- Calf blankets/coats/jackets help prevent heat and energy loss, especially to the younger and smaller body weight calves.

No matter the weather, remember the 5 "C's" to calf care and diligently follow the basics regarding getting calves off to a good start.

- Colostrum
- Consistency
- Comfort
- Calories
- Cleanliness

It is also encouraged to revisit calf health protocols to ensure calves are receiving the best care we can provide them:

- Vaccination protocols for dams and calves.
- Provide a clean calving area and prompt removal of calf from dam.
- Dip navel with either Chlorhexidine solution or a 7% Tincture of Iodine.
- Practice good colostrum management, determine if calves are receiving adequate levels of passive immune transfer.
- Dry calf hair coats to prevent heat loss.
- Minimize exposure to moisture and manure in calf housing – provide clean, dry bedding.
- Provide adequate ventilation.
- Clean calf feeding equipment daily – bottles, nipples, buckets, etc.

upon us, remember to encourage producers to spend a little more time and effort on the pre-weaned calves so that they can fulfill their potential to be the great milk producers of tomorrow.

## Virology

### Diagnostic / Surveillance testing:

- The first confirmed case of Eastern equine encephalitis (EEE) for 2019 was detected on July 31, 2019 from a quarter horse mare in Barron Co. The horse had not been vaccinated for EEE and showed neurologic signs. On September 24, 2019, a horse from Waukesha Co. also tested POS for EEE. As a reminder when submitting samples for EEE testing, please provide information on whether or not the animal is exhibiting neurological signs, a complete vaccination history and if the animal has traveled outside the state of Wisconsin.
- The West Nile Virus (WNV) testing season is winding down for 2019. WVDL partners with public health to test skin samples from members of the Corvidae family (crows, ravens, and blue jays). Testing to date is below:

#### 2019 WNV Positive County Report - 9/20/19

Total Tested	Positives	Negatives	Counties Tested	Counties Closed	American Crow	Blue Jay	Common Raven
36	2	34	22	2	21	11	4

- In April 2019, WVDL was notified that Veterinary Diagnostic Technology, Inc., the source of our BTv, EHD and BLV AGID kits as well as the BTv cELISA kit will no longer be in business. We are continuing to work with USDA and collaborators to find and validate an acceptable alternative test.

**NAHLN (National Animal Health Laboratory Network):** On Sept 23-24, WVDL participated in a tabletop African Swine Fever (ASF) outbreak scenario exercise. The exercise was designed to introduce an escalating outbreak of ASF in North America and address a variety of injects over several days. Specific goals were to determine the following:

- Provide an opportunity for laboratories to exercise their capability to enact enhanced biosafety protocols, and identify any resource gaps impacting this.
- Practice coordination of communications between the laboratory and various entities involved with the laboratory during an outbreak situation.
- Practice sample log in, preparation, testing and result messaging.
- Identify resource gaps for outbreak response at the biosafety/biosecurity, technical, and laboratory policy levels.

## Pathology Sciences

### Seats are Available for the 2019 Veterinary Forensics Seminar and Autopsy Wet Lab

- **Sunday, Nov. 3 - 8:00 a.m. - 5:00 p.m.**

WVDL will be sponsoring a veterinary forensics seminar and wet lab on Sunday November 3<sup>rd</sup> from 8am-5pm. This year's chosen speaker will be Dr. Melinda Merck who is a leader in the veterinary forensics field. She will provide a wealth of knowledge and an engaging experience for all those in attendance. The goal of this seminar is to provide training in an environment that promotes cohesiveness and teamwork in forensic casework amongst the target audience while enhancing their knowledge and skills in handling animal cruelty cases. The target audience for this seminar includes but is not limited to: Wisconsin DVMs, CVTs, SVM students, animal welfare groups, animal control, and law enforcement. The link to more information and registration is below; the seats will be limited and the wet lab is restricted to only 25 participants so registering as soon as possible is recommended.

The link to our registration is: <https://apps.vetmed.wisc.edu/cereg/>

### Rendering companies no longer accepting horses

In a recent letter from Darling Ingredients they stated, "Due to risks associated with horses that may have been euthanized with pentobarbital we can no longer accept horses or any horse parts, effective immediately". The

### Increase in Disposal Charges

We have done our best to keep the cost of carcass disposal to our clients very low over the years. Due to increases in costs over the last few years, we are raising the rates of our animal disposal options. We implemented a cost increase on July 1<sup>st</sup>, 2019 and began charging clients \$1.50 per pound for alkaline hydrolysis tissue digestion and \$6.00 per 100 pounds for rendering.

Additionally, the cost of disposing our regulated medical waste and pathological waste via incineration has increased dramatically in the last year. We will be increasing the cost charged to clients to \$1.00 per pound beginning on November 1st.

Disposal method	Previous price / lb	Barron-New price / lb	Madison-New price / lb
Digestion	\$0.50	NA	\$1.50
Incineration (Madison)	\$0.50	\$0.50	\$1.00
Rendering	\$0.00	\$6.00 / 100 lbs	\$6.00 / 100 lbs

### Lab space re-allocation update

WVDL has completed the transfer of equipment and instrumentation between the Chemistry and TSE labs. The result of this re-allocation of lab space will increase the footprint of the TSE lab by nearly three times. We also invested in 2 new Biosafety Cabinets and an additional tissue homogenization instrument to increase our capacity. This fall, we are expecting approximately 40,000 samples for CWD ELISA testing. The [Cap Times](#) recently printed a story about the WVDL TSE Testing Lab.

### Forensic/Legal Necropsy at WVDL

- *Clarification of who may request and have forensic/legal necropsies performed at WVDL.*
1. Police / Law enforcement officers within the state of Wisconsin.
  2. District attorneys, government, within the state of Wisconsin, usually in conjunction with police / law enforcement officers within the state of Wisconsin.
  3. Licensed Veterinarians within the state of Wisconsin in conjunction with police / law enforcement officers and/or district attorneys, government, within the state of Wisconsin, and or Animal Control Officers within the state of Wisconsin.
  4. Animal Control Officers / Humane Society personnel within the state of Wisconsin, working in conjunction with police / law enforcement officers or district attorneys, government, within the state of Wisconsin.
  5. Wisconsin Department of Natural Resources (DNR) personnel performing official work, in conjunction with DNR legal/law enforcement, or police / law enforcement officers or district attorneys, government, within the state of Wisconsin.

### Other categories of necropsies currently performed at WVDL

- Routine: Submitted by licensed referring veterinarians.
- Insurance: Submitted by licensed referring veterinarians.

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