



Wisconsin Veterinary Diagnostic Laboratory

UNIVERSITY OF WISCONSIN-MADISON

2020 Fall Newsletter

Message from the Director

Greetings from the WVDL! We all hope that you and your families continue to stay safe. Despite all of the challenges our profession and the State of Wisconsin is enduring, the WVDL is functioning quite well to meet your needs. Caseload has been returning to pre-COVID levels and our testing turnaround times are holding steady. Because of the negative effects of COVID on the beef and dairy industries, we did not increase fees for individual diagnostics for clinical management of cattle diseases. We hope that this provides some relief to farms and welcome any feedback on this decision. We are expecting an uptick in EIA and biopsy submissions with anticipated changes in availability and pricing for veterinary diagnostics in the new year. Please contact us if your needs or services have changed to explore options at WVDL.

CWD testing is expected to increase this year with 24,000 tests from the WI DNR and an additional 50,000 tests from out of state contract testing. We also just completed an exercise with the USDA to work on validation of bulk tank milk as a diagnostic sample for FMDV during an outbreak. This seemed like it should be a simple process, but we have found that in fact, it is not.

SARS-CoV-2 testing in animals is holding steady and most of our work is for a collaborative project with the CDC and for mink farms in Wisconsin. WVDL is in a joint partnership with WLSH to perform human COVID testing with a goal of 12,000 to 15,000 tests per week by the end of 2020, holding steady until June of 2021. This will not handle all of UW-Madison or UW System's testing needs on a daily basis, but will instead be used strategically as the gold standard in collaboration with other testing strategies.

WVDL is excited to announce the arrival of a new pathologist, Dr. Andrea Pohly, and a new financial manager, Paul Benedict. Welcome Andrea and Paul!

As always, please contact us at any time with diagnostic, case management, or infectious disease related questions.

Keith Poulsen, DVM, PhD
Director



Salmonella Brandenburg Abortion in Dairy Heifers

• A Recent Case Study

In the first week of August, during a span of 7-8 days, a commercial Illinois dairy received approximately 250 Holstein dairy heifers that were six months pregnant from a commercial heifer raiser located in Colorado. Heifers were transported directly from Colorado to Illinois. The abortions of these heifers started 3-5 weeks after the animals arrived at the dairy and have continued since that time. The number of abortions peaked during the last week of August and the first two weeks of September. To date, there have been 25-30 abortions with the gestational age of the fetuses ranging from 200 – 230 days.

The WVDL received fresh and formalin-fixed tissue samples that were collected from five aborted fetuses. The submitting veterinarians requested routine diagnostic work up for bovine abortions. *Salmonella* was isolated by bacteriological culture from tissue samples collected from all five fetuses with MALDI-TOF confirmation of the isolates. No other known abortifacient agents other than *Salmonella* were found. *Salmonella* serotyping using the Kauffman and White classification scheme identified the *Salmonella* isolates as *Salmonella enterica* serovar Brandenburg, a serogroup B *Salmonella*. Serotyping on the first diagnostic submission was completed by WVDL microbiologists seven business days after receiving the samples.

In the past 15 years, the WVDL has found *Salmonella* ser. Brandenburg in cattle twenty-seven times with some of the isolates coming from bovine fetuses. The significance of this particular herd abortion scenario is striking as the number of 3rd trimester abortions (25-30) that were probably caused by *Salmonella* ser. Brandenburg.

In our experience, bovine abortion storms caused by a *Salmonella* spp., are exceedingly rare. The source of *Salmonella* ser. Brandenburg that caused the abortion outbreak has not been determined. It can be very difficult to determine where the original source of infection came from because one or more carrier animals can rapidly contaminate the environment which includes community water tanks.

Similar to the WVDL findings, New Zealand has reported abortion outbreaks caused by *Salmonella* ser. Brandenburg in both sheep and dairy cattle.¹ Dairy practitioners should consider *Salmonella* ser. Brandenburg as a potential cause of abortions if ≥ 5% of first calving dairy heifers abort in the 3rd trimester of pregnancy.²

Further information on the WVDL's *Salmonella* PCR and Culture testing work-flow can be found on our *Salmonella* page of the WVDL website. Please visit this link to learn more information regarding this information as well as more topics regarding *Salmonella*. Link: <https://www.wvdl.wisc.edu/index.php/salmonella/>

1. RG Clark, SG Fenwick, CM Nicol et al. 2004. *Salmonella* Brandenburg-emergence of a new strain affecting stock and humans in the South Island of New Zealand, New Zealand Veterinary Journal, 52:1, 26-36.
2. EL Cuttance & on behalf of the *Salmonella* Brandenburg Investigation Group (2018). A case definition for *Salmonella* Brandenburg infection in dairy heifers, New Zealand Veterinary Journal, 66:1 52-52.

Microbiology

The WVDL performs a variety of testing modalities for bovine respiratory disease (BRD). These include BRD panels that are molecular testing (PCR) alone, bacterial culture alone or both (PCR and culture). Depending on what testing modality is selected, particular transport media is needed as some types of media interfere with the testing modalities being requested. Please see our website for more information on testing options and media that corresponds to the specific testing being requested. If further questions or concerns about sample collection and transport media to select arise, please feel free to contact the lab for confirmation. The following links to the respiratory disease submission form and sampling techniques are available on the WVDL website:

- <https://www.wvdl.wisc.edu/wp-content/uploads/2020/09/BovineRespiratoryPanel.pdf>
- <https://www.wvdl.wisc.edu/wp-content/uploads/2020/09/Use-of-Deep-Nasopharyngeal-Swabs.pdf>

Below is a limited analysis of the BRD pathogen results obtained in 2019 for both molecular testing and bacterial culture. The WVDL Virology Section evaluated 1,403 samples for bovine respiratory viruses and bacteria (**Table 1 and 2**) using real time PCR assay. The greatest percentage of positive samples were bovine coronavirus (23.35%) for viral agents and *Pasteurella multocida* (41.91%) for bacterial agents. Compared to 2018 data, the greatest reduction in PCR positivity rates were seen for bovine herpesvirus-1 (IBR, -61.67%) as well as for bovine viral diarrhea virus (BVD, -12.50%).

The Bacteriology Section evaluated 1,640 samples (**Table 3**), where the greatest percentage of positive cultures were for *Pasteurella multocida* (22.62%) or *Mannheimia haemolytica* (9.88%). For bacterial culture, reductions were seen in the positive rates for *Escherichia coli* (-44.9%) and *M. haemolytica* (-17.33%). Whereas, increases were seen for *Bibersteinia trehalosi* (43.12%), though the sample size was limited, *Truiperella pyogenes* (31.35%) and *Salmonella enterica* subspecies *enterica* serotypes (27.45%). Additionally, the WVDL performs antimicrobial susceptibility testing using minimum inhibitory concentration (MIC) methods following the Clinical Laboratory

Standards Institute (CLSI) breakpoints for defining susceptible, intermediate and resistant microbes (**Table 4**). Only those organisms with respiratory-approved CLSI breakpoints are listed below, in which *Mycoplasma bovis* nor *B. trehalosi* has no CLIS breakpoints. No significant changes were observed from 2018 and BRD pathogens remain relatively susceptible to the approved antimicrobial agents.

The WVDL does not perform serology for BRD pathogens and generally does not recommend serology as molecular and culture detection is considered more sensitive. Additionally, vaccination for these pathogens makes interpretation difficult.

Table 1 Real time PCR positive samples for bovine respiratory disease viral agents (1,403 samples)

<u>Respiratory Pathogen</u>	<u>No. Positive</u>	<u>% Positive</u>	<u>Percent Change from 2018</u>
Bovine respiratory syncytial virus	120	8.55%	-10.74%
Bovine coronavirus	318	23.35%	5.97%
Bovine viral diarrhea virus	16	1.17%	-12.50%
Bovine herpesvirus-1	34	2.50%	-61.76%

Table 2 Real time PCR positive samples for bovine respiratory disease bacterial agents (1,403 samples)

<u>Bacterial Pathogen</u>	<u>No. Positive</u>	<u>% Positive</u>	<u>Percent Change from 2018</u>
<i>Histophilus somni</i>	290	20.67%	No data
<i>Pasteurella multocida</i>	588	41.91%	No data
<i>Mannheimia haemolytica</i>	285	20.31%	No data
<i>Bibersteinia trehalosi</i>	134	9.55%	No data
<i>Mycoplasma bovis</i>	411	30.18%	7.79%

Table 3 Culture positive samples for bacterial BRD pathogens (excluding *Mycoplasma*, 1,640 samples).

<u>Bacterial Pathogen</u>	<u>No. Positive</u>	<u>% Positive</u>	<u>Percent Change from 2018</u>
<i>Histophilus somni</i>	120	7.32%	10.98%
<i>Pasteurella multocida</i>	371	22.62%	19.34%
<i>Mannheimia haemolytica</i>	162	9.88%	-17.33%
<i>Bibersteinia trehalosi</i>	29	1.77%	43.12%
<i>Salmonella</i> species	118	7.20%	27.45%
<i>Gallibacterium anatis</i>	15	0.91%	26.69%
<i>Trueperella pyogenes</i>	127	7.74%	31.35%
<i>Escherichia coli</i>	90	5.49%	-44.87%

Table 4 Percent susceptibility of each BRD microorganism to the antimicrobials listed using the Clinical Laboratory Standards Institute (CLSI) breakpoints for minimum inhibitor concentrations.

<u>Antimicrobial</u>	<u><i>P. multocida</i></u>	<u><i>M. haemolytica</i></u>	<u><i>H. somni</i></u>
Ampicillin	98%	93%	93%
Ceftiofur	97%	100%	98%
Danofloxacin	95%	98%	NI
Enrofloxacin	93%	86%	94%
Florfenicol	93%	91%	100%
Gamithromycin	81%	83%	88%
Penicillin	94%	78%	95%
Spectinomycin	81%	92%	87%

Tetracycline	67%	80%	85%
Tildipirosin	80%	88%	88%
Tilmicosin	77%	80%	NI
Tulathromycin	87%	89%	75

NI- Not interpreted due to no CLSI breakpoint

Ovine Sera Approved for Johne's Disease Antibody ELISA

The WVDL has performed an internal evaluation of ovine sera for Johne's antibody disease diagnostics using two commercially available kits. The VMRD *Mycobacterium avium* subsp. *paratuberculosis* (MAP) Antibody Test Kit performs well with the highest sensitivity and specificity between the two kits tested. This ovine sera is now accepted for validated ELISA testing and will have the same S/P cut-off as caprine sera. This cut-off is different than for bovine sera and milk. ELISA results work well for surveillance and the WVDL encourages any positives to be confirmed using the MAP PCR on fecal samples, which utilizes three PCR targets for superior sensitivity. For more information about Johne's Disease testing at the WVDL please refer to <https://www.wvdl.wisc.edu/index.php/johnes-disease-diagnostics/> or feel free to contact the laboratory to address your questions and concerns.

Important Information Regarding Selection of Isolates for Antimicrobial Susceptibility Testing

The bacteriology laboratory commonly runs the minimum inhibitory concentration (MIC) method for antimicrobial sensitivity testing on enteric, respiratory and mastitis causing pathogens. Antimicrobial susceptibility testing is routinely performed on samples submitted through necropsy services, but also is performed by request from the submitting veterinarian for samples such as deep nasopharyngeal swabs, milk, and fecal samples. The WVDL routinely sets aside at least one type of pathogen isolate per accession, but cannot save all isolates from all animals in an accession. For example, if 5 *Pasteurella multocida* and 2 *Mannheimia haemolytica* isolates are isolated, the WVDL will do 1 *P. multocida* and 1 *M. haemolytica* antimicrobial susceptibility tests.

If you are submitting samples for diagnostic work-up where the submission includes multiple animals, of various ages, we recommend the following when more than 1 susceptibility on the same pathogen isolated from different animals is needed.

RECOMMENDATION: Please indicate on the submission form or let your case coordinator know that you would like antimicrobial susceptibility testing conducted for the particular animals or pathogens of concern. Remember that for EACH isolate there will be an individual antimicrobial susceptibility charge (Example – 5 *Pasteurella multocida* isolates will have 5 individual antimicrobial susceptibility testing charges). The cost of this antimicrobial susceptibility testing is dependent on in-state or out-of-state status.

IMPORTANT: If this information is requested upfront and there is knowledge of further susceptibility testing, the more likely the lab will be able to have the isolates available and MIC values returned to you in a timely fashion. Thank you for your cooperation and please feel free to contact one of our case coordinating veterinarians on staff if further questions and/or concerns arise.



Staff Spotlight:

Andrea Pohly, Pathologist

1. Where are you from? What high school and college did you graduate from? What are your degree(s) in?

I am from Cleveland, Ohio and went to Laurel High School. I attended Kenyon College (Gambier, Ohio), where I majored in Biology before receiving my DVM and Master of Science degree from the University of Illinois. My residency in Veterinary Anatomic Pathology was also completed at UI.

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

To hold a baby orangutan

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

- Jubb, Kennedy and Palmer's Pathology of Domestic Animals. (I'm currently studying for boards because it had previously been cancelled due to COVID-19). But, I just finished Where the Crawdads Sing.

4. **What is the one food that you will never bring yourself to eat?**
 - Apples ... the texture and flavor are a "no" for me.
5. **What is your favorite movie soundtrack?**
 - Harry Potter.
6. **If you could play the lead role in any movie, which character would you choose?**
 - Anything with Tina Fey or Amy Poehler.
7. **What three flavors of ice cream would be on your triple scoop cone?**
 - Chocolate, Chocolate and Chocolate.
8. **When you were a child, what or who did you want to be when you grew up?**
 - A veterinarian ... how original!!!!.
9. **What pets did you have growing up?**
 - A first dog named Jeff (don't let your 5-year old name your pets like my parents did). A second dog named Rosie (my older brother named this one after the 'Jeff' incident). Many hamsters, fish, a cockatiel, a rabbit, lizards, frogs, chickens. You name it, we probably had it at some point.
10. **What skill would you like to master?**
 - Baking!
11. **What is your biggest pet peeve?**
 - Talking during movies or when watching TV
12. **Best concert you ever saw?**
 - Garth Brooks
13. **If you could be any animal in the world, what animal would you be and why?**
 - A dog because they eat and sleep all day and are treated like royalty in many households (including my own).
14. **What led you down the career path you are on?**
 - I actually didn't know veterinary pathology existed and I was attempting to get into vet school. I shadowed the Animal Disease and Diagnostics Laboratory in Ohio and found it fascinating. I never knew that there were veterinarians on this side of medicine that were involved in herd health and public health and I thought that the interface between human and animal disease was something that I wanted to be a part of!! Eight years later, here I am!!

Virology & Molecular Diagnostics

Diagnostic & Surveillance Testing Update:

Testing schedule modifications: Recently the Virology section at the WVDL modified our testing schedule. These modifications were mainly due to type and number of samples received and workload changes at the WVDL in the COVID era. Bovine Viral Diarrhea Virus (BVD) PCR now routinely utilizes a robotic platform which enables 384 samples to be tested in one PCR run at a time. This testing capability allows us to maximize reagents as well as microbiologist time and efficiency.

As a reminder, for samples to make the scheduled assay testing they need to arrive at the lab by noon the day prior to testing day, so it can be processed and setup on schedule. If the sample arrives after the noon deadline it will be setup for the next scheduled assay testing day.

Below are the assay schedules that have changed:

Assay	Testing days**
BLV ELISA	M, W, Th, F
BTV ELISA	M, W, F

IBR ELISA	M, W, F
BVD ACE	M
BVD PCR (EN's, swabs, whole blood)	Tu, Th
Enteric PCR panel	Tu, W, Th, F
BLV & Anaplasma PCR	F

**M= Monday, Tu= Tuesday, W= Wednesday, Th= Thursday, F= Friday

Virus Headlines: EEE & SARS-CoV-2

Recent EEE Cases

The first confirmed case of **Eastern equine encephalitis (EEE)** in Wisconsin for 2020 was detected on July 23, 2020 from a 10-year-old mare in Dunn County. The horse had not been vaccinated for EEE and had clinical neurologic signs when evaluated. Since this first positive case, 4 additional equine cases have tested PCR-positive from postmortem CNS tissues.

Additionally, 9 antemortem serum samples from horses with clinical neurological signs have tested positive on the EEE IgM capture ELISA performed at the National Veterinary Services Laboratory (NVSL) in Ames, Iowa, suggesting a recent exposure to EEE virus.

EEE is a zoonotic disease that is transmitted by mosquito vectors to animals and humans. With recent and upcoming frost weather events these mosquito reservoirs will be reduced for this season. Prevention by vaccination and boosting is HIGHLY recommended for horses (www.aaep.org), especially in areas that have a high incidence of disease. **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, as well as information regarding the animal's travel history, especially if outside the state of Wisconsin.**

SARS-CoV-2 PCR testing in animals

Since April 2020, WVDL has validated a real-time PCR assay for diagnostics testing of SARS-CoV-2 in veterinary species. Our real-time PCR is based on the N1 and N2 PCR assays published by the Centers for Disease and Control (CDC). The WVDL has been in collaboration with CDC and NAHLN to provide diagnostic testing for samples collected from companion animals in COVID-19 positive households and animal shelters, which have had close interactions with owners or caretakers. The WVDL has been granted a one year contract from the CDC to continue testing under this collaborative framework.

The guidelines and resources for SARS-CoV-2 testing in animals are available on the WVDL website at the following link: <https://www.wvdl.wisc.edu/index.php/wisconsin-veterinary-diagnostic-laboratory-faqs-during-covid-19-interruption>. While routine testing of sick animals is not warranted under current testing guidelines, the WVDL will accept SARS-CoV-2 PCR testing based on an epidemiologic need. If sample submission for SARS-CoV-2 testing on live animals is requested for testing at the WVDL, please contact the WVDL prior to sample submission. We advise that submitting veterinarians need to collect 3 swabs if at all possible in the following priority order: **nasal, oral, rectal**. These samples should be collected in BHI (Brain Heart Infusion) broth, otherwise PBS (Phosphate Buffered Saline) or saline can be used as an alternative. If collection media is needed please contact the WVDL for ordering swabs and transport media.

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