WVDL Quarterly Newsletter - Summer, 2021

Wisconsin Veterinary Diagnostic Laboratory
UNIVERSITY OF WISCONSIN–MADISON

2021 Summer Newsletter

Message from the Director

Greetings from WVDL! We hope you are all enjoying a summer that is vastly different from 12 months ago! We have quite a bit of news to share with you from our Madison and Barron facilities and want to thank you for your time to catch up with our teams.

There are two case reports to read about, new tests, tips and tricks to improve your diagnostic testing results, new antimicrobial sensitivity testing guidelines, and an extensive review of *Samonella* isolates from 2019 and 2020. Don't miss this edition's staff spotlight on Melissa Lund, our new Serology supervisor.

We are happy to announce that our fee increase for the 2022 fiscal year will be 2%, decreased from the previously announced 3%. We are also excited to drop the $10 accession fee for accessions submitted electronically with our new database system (iLES), which is being deployed on September 1, 2021. We will have resources on our website and will offer training, as needed, to help your practice submit and retrieve results as we significantly upgrade our electronic information management systems.

Our summer bovine genetics export meeting will be held on 7/21/2021 as a hybrid in-person and virtual conference. This meeting is free and geared towards diagnostic testing for export of bovine germplasm. This meeting will have 6 hours of CE. If you are interested in attending and have not registered, please email or call.

Lastly, we are very excited to have an extensive renovation and remodeling project for the Barron facility approved in the recently signed biennial budget for the State of Wisconsin. I would like to thank all of our stakeholders and clients that helped make this project a reality.

Enjoy the rest of your summer and On Wisconsin!

Keith Poulsen
Director

Corynebacterium mustelae Endocarditis in a Dog

Alexandra Harvey, Christine Watson, Beth Angell, Nicole Aulik and Lorelei Clarke
Wisconsin Veterinary Diagnostic Laboratory, University of Wisconsin-Madison, Madison, Wisconsin
Journal of Comparative Pathology (2021) May 185:82-86. Full text here.

Recently, the WVDL pathology and bacteriology departments teamed up and were able to publish an article

entitled "*Corynebacterium mustelae* Endocarditis in a Dog" in the Journal of Comparative Pathology. This publication describes a case of *Corynebacterium mustelae* isolated from tissue in a canine postmortem exam with correlating gross and histopathology. There is only one previous report of this agent being associated with endocarditis in dogs. This organism is a facultative anaerobe and was difficult to identify through standard culture methods, possibly indicating that other fastidious organisms may play a role in canine endocarditis cases. This case also demonstrates the value of a thorough post-mortem workup and the value of communication between clinicians, pathologists, and microbiologists. Please see the abstract below.

Abstract:
A seven-year-old male neutered Labrador Retriever dog presented with acute onset fever, shifting limb lameness, and anorexia with development of acute respiratory distress. On postmortem exam, there was vegetative endocarditis effacing the aortic valve. Gram stains of impression smears from the aortic valve and kidney revealed numerous gram-positive rods with some coryneform bacteria. Similar coryneform bacteria were isolated on aerobic culture of the aortic valve. Identification was attempted by MALDI-TOF and 16S sequencing, the latter of which indicated *Corynebacterium mustelae*. This is the second reported case of endocarditis in a dog involving *C. mustelae* and the first with postmortem pathology. This case serves as an example of the utility of various modalities to identify facultative anaerobic bacterial pathogens that may be difficult to culture and may be more widespread than previously diagnosed.

Salmonella Typhimurium Outbreak in a Small Dairy Herd

D.C. Sockett DVM, MS, PhD, Diplomate ACVIM-LA

In early June 2021, the WVDL received fecal, water, feed and tissue samples from aborted fetuses from an 80 cow dairy herd. The affected lactating cows were febrile (103.5-104.8°F), exhibited signs of watery diarrhea, anorexia and significantly reduced milk production. All 80 cows became ill in a period of 1-3 days. Cows showing signs of illness were treated with systemic antibiotics and approximately 40 cows were administered IV hypertonic saline combined with oral electrolyte fluids. Milk production was reduced by at least 90% across the herd. Six lactating cows died and 17 cows aborted a 3 to 6-month-old fetus. None of the cows within the herd were vaccinated with a commercially available *Salmonella* vaccine.

The herd was housed in an old tie stall barn that had been converted to a free stall barn. Ambient daytime temperatures during this outbreak were between 93-95°F with a relative humidity of at least 70%. The heat index was 116-124°F. Heat abatement procedures had not been in place to cool cows prior to the outbreak. The herd veterinarian instructed the dairyman to install a number of large fans for heat abatement during this unseasonably warm weather.

Multiple samples submitted to the WVDL and the bacteriology group isolated a multi-drug resistant *Salmonella* Typhimurium. Sample types included feces, aborted fetuses, feed, and water from the communal water tanks that the lactating cows had access to and were drinking from. *Salmonella* was not isolated from the submitted feed (TMR) samples. Instructions to the dairyman were to clean and sanitize the water tanks plus provide gut microbiome support to his cows by adding a commercial probiotic to the TMR. The advised probiotic that was used has proven efficacy (publicly available data) against *Salmonella* Typhimurium. Recommendations to initiate a rodent control program were also given since rodents are the maintenance host for *Salmonella* Typhimurium.

Cows at this dairy were exposed to a very invasive serotype of *Salmonella* Typhimurium. The increased presence of *Salmonella* Typhimurium in the drinking water along with heat stress, exacerbated morbidity and mortality within this herd. Dairy cows experiencing heat stress will increase their daily water consumption by 50-100%. This markedly increases the daily number of *Salmonella* bacteria cows have exposure. Research has also shown that heat stress causes "leaky gut syndrome" which easily allows pathogenic bacteria to leave the gut causing bacteremia in affected animals.

When confronting herd health outbreaks with increased morbidity and mortality to the animals within the herd, do not forget to overlook different point sources on the premise. Please feel free to contact the WVDL during herd health outbreak investigations to discuss available testing, appropriate sample types, and possible interventions to improve animal health.

Molecular Diagnostics

Johnes Fecal PCR Testing

In an effort to provide excellent customer service, we would like to communicate tips on submitting samples for Johne's disease fecal PCR testing. Since we are an accredited laboratory, we are required to follow certain guidelines set forth by our quality program and accrediting bodies. We understand that our clients must adhere to their set guidelines and protocols and want to ensure high quality diagnostic results.

Sample submission requirements/recommendations:

1. Samples submitted must be identified on the submission form. This information should include the identification information that is written on the sample vials for traceability.
   NOTE: We recommend only listing the samples that are being submitted for testing.

2. A composite (a sample that is not from a single animal) can be submitted, but please label with a term such as composite rather than pool as this can create confusion when other samples on the same submission form might need to be pooled.

3. WVDL is limited to pooling up to 5 samples per pool. Individual samples may be from different animals or different sample days from one animal. However, composite samples cannot be pooled with any other samples, as this reduces sensitivity. Pooling of individual animal samples will occur at the WVDL, so that individual animals in positive pools can be tested separately and run to identify the source of a positive result.
NOTE: Reminder that any positive PCR pool that represents different animals will be automatically un-pooled and tested individually to determine the positive sample.

4. It is helpful to our staff if samples to be pooled could be listed on the submission form together and then individual samples grouped together. An example would be listing pooled PCR samples as samples 1-10 and then individual PCR as samples 11-15.

5. WVDL has fecal collection vials available free of charge. These can be ordered by filling out this order form and faxing or emailing to our client services team.

Johnes PCR test interpretation
At the WVDL, we use an in-house validated Direct Fecal PCR that detects Mycobacterium avium subsp. Paratuberculosis (MAP) extracted from fecal samples using three different gene targets including IS900, the most recognized gene target for MAP. An interpretation protocol has been established based on over 2,500 diagnostic samples of multiple different species in order to utilize these three targets to provide the most comprehensive MAP detection available.

In order for a sample to be considered positive, two of the three targets must be detected in the multiplex PCR in which one must be IS900. The IS900 target Ct is the value that is reported on the result report since this target is the most sensitive due to being present in multiple copies in the genome. The validation completed at WVDL has shown that the combined use of these three targets in one assay is a reliable tool for MAP detection and limits cross-reactions from other members of the Mycobacterium avium complex.

For a small subset of single samples (less than 0.01%), particularly for exotic samples, the interpretation using a three-target multiplex PCR could not accurately be determined. This data, combined with the animal histories, required us to create a new test interpretation of “undetermined”. The test result of undetermined will be entered on a result report based on our validation criteria and WVDL recommends retesting this animal within six months.

For more information on Johnes testing interpretation and the assays that are offered; visit the diagnostic aide section of our website.

Bovine Scours Panels
WVDL offers three scours panel PCR testing options to aid in the detection of the 5 major bovine enteric pathogens. Each of the assays may be ordered as a panel or as individual assays. All individual PCR assays and panels require a minimum of 0.5g of fecal/intestinal material. The assays are run on Monday, Wednesday and Fridays with results available the next day. Please be sure to keep the samples cold and ship on freezer packs to maintain sample integrity. DO NOT send fecal samples in gloves. Veterinarians should completely fill out the WVDL Bovine Enteric Disease Panel submission form found on our website.

Scours Panel A: This panel includes the 3 most common infectious agents found in neonatal calf scours; bovine rotavirus, bovine coronavirus and cryptosporidium parvum.
Scours Panel B: This panel is recommended for calves ***7 days of age or older*** and includes bovine rotavirus, bovine coronavirus, cryptosporidium parvum and *Salmonella* spp*.
Scours Panel C: This panel is recommended for calves l***ess than 7 days of age*** and includes bovine rotavirus, bovine coronavirus, cryptosporidium parvum, *Salmonella* spp*, and E. coli K99 and intimin genes.

**Salmonella* spp PCR requires an overnight incubation at the laboratory before PCR assay can be performed. All *Salmonella* spp. PCR results that yield a CT value of less than or equal to 35, will automatically be sent for culture and serotyping. If you do NOT want these additional tests performed or only want one of these tests performed, please contact the WVDL. For more information please see our website for a full description of our *Salmonella* workflow.

Serology

Q-Fever ELISA to start August 1, 2021 and Replaces the Q-Fever Complement Fixation (CF) Test
Starting August 1, 2021 the WVDL will be replacing the aging Q-Fever Complement Fixation (CF) test with the Q-Fever ELISA. The Q-Fever ELISA has a sensitivity of 83% and specificity of 94% without an inconclusive range. However, the WVDL has developed an inconclusive range that brings the sensitivity and specificity to 100%. For inconclusive results, we recommend retesting or referring to NVSL. We will provide the Q-Fever CF until September 1 for any diagnostics that must be performed. Please plan to shift your testing from the Q-Fever CF to the Q-Fever ELISA. The cost of the test is $15 per sample.

***Salmonella* Dublin Antibody ELISA**
The ThermoFisher PrioCHECK *Salmonella* Dublin Antibody ELISA has now been validated for milk and bulk tank milk along with serum and plasma. This ELISA can be used as a biosecurity screen when purchasing animals or for surveillance in existing herds. A positive test result from an individual bovine animal indicates

the presence of antibodies, which suggests that the animal has been exposed to, or is a carrier of, *Salmonella* ser. Dublin. An animal should be ***greater than 100 days of age*** for this test to be accurate. A negative test result indicates the lack of antibodies and thereby a lack of exposure to *Salmonella* ser. Dublin, or there was an exposure that resulted in antibody production below the limit of detection for this test.

Individual animal management should not be based solely on an ELISA test results. As with any biological test, occasional false positives or negatives do occur as a result of local conditions with the animal, herd or location. Seroconversion can take up to 7 weeks following infection. A test should be interpreted in the context of all available clinical, historical, and epidemiological information. For further testing of animals with clinical symptoms, the WVDL offers a PCR test for both *Salmonella* species and *Salmonella* ser. Dublin. A carrier animal is defined as those that remain positive for three ELISA tests over an 8 to 12 month period (LR Nielsen et al., 2004). An ELISA-positive animal may not yield a positive fecal PCR result as some animals shed *Salmonella* ser. Dublin at an undetectable level and therefore PCR cannot be used to rule out the possibility of the animal being a carrier. Please see our website for more information. The price is $9.55 per sample and is available at WVDL-Madison.

Caseous Lymphadenitis (CL) ELISA Testing Available at WVDL-Barron
Along with bacterial culture for *Corynebacterium pseudotuberculosis* at both the Madison and Barron WVDL locations, the Barron WVDL also offers CL (occasionally referred to as CLA) ELISA. This is a serological assay to detect *C. pseudotuberculosis*-specific antibodies in sheep and goat sera. For definitive diagnosis of animal showing clinical signs (visible subcutaneous abscesses), please submit abscess material for culture to directly diagnose CL. However, for herd level screening, please use the CL ELISA.

REMINDER: a serological test for an individual animal should be interpreted with caution given the animal could have an active or past infection that has resolved. The CL ELISA should be used to determine herd prevalence of the infection. The CL ELISA costs $9.00 per sample. In addition, the WVDL-Barron location offers Ovine Progressive Pneumonia (OPP) Virus ELISA as well as the Caprine Arthritis Encephalitis (CAE) virus ELISA, and the Madison location offers Johne's disease diagnostics including ELISA, culture and PCR. Please submit at least 0.5 mL of serum.

Staff Spotlight: Melissa Lund, Serology Supervisor

Where are you from? What high school and college/university did you graduate from?
I grew up in many places around the world so I don't have a specific place that I like to say that I am from. However, I was born in Florida, graduated high school in Ohio. I attended UW-Madison and graduated with degrees in Biology and Wildlife Ecology

What is one thing on your 'Bucket List'?
To see the northern lights with my family

What book(s) are you currently reading?
Just finished "Being Mortal" by Atul Gawande. Getting ready to start "Talking to Strangers" by Malcolm Gladwell

What is the one food that you will never bring yourself to eat?
Liver. That's a whole lotta nope.

What is your favorite movie soundtrack?
A tie between Forest Gump and Captain Marvel

If you could play the lead role in any movie, which character would you choose?
Ellen Ripley from the Alien franchise

What three flavors of ice cream would be on your triple scoop cone?
Chocolate Peanut butter
Zanzibar Chocolate (from Chocolate Shoppe)
Chocolate Chocolate chip

When you were a child, what or who did you want to be when you grew up?
Dr. Jane Goodall or Wonder Woman (still working on Wonder Woman)

What pets did you have growing up?
Cats, dogs, hamsters, zebra finches, cockatiel, fish

What skill would you like to master?
Playing the ukulele. It's one of the few instruments that always sounds a little happy regardless of the song. I have one but haven't had the time to sit and learn….yet.

What is your biggest pet peeve?
Non-attentive or erratic drivers

Best concert you ever saw?
A concert with the Goo Goo Dolls/Counting Crows/Matchbox 20/Garbage line-up. I think I'm dating myself on

the music questions.

If you could be any animal in the world, what animal would you be and why?
River or sea otter because they always look like they are living their best life.

What led you down the career path you are on?
I've always had an interest biology, particularly the environmental science and wildlife. However, over time I took a deeper interest on the microbiology particularly immunology. And now I'm here!

Virology

Help us help you with diagnostic testing!

BVDV Testing

EAR NOTCH SAMPLES
WVDL receives thousands of ear notch samples each year. As a reminder, below are sample collection guidelines. Please see our website for complete details as well as guidelines for nasal swab collection for BVD PCR testing here.

Ear notch sample collection guidelines:

1. Materials and equipment needed for ear notch collecting:
    1. Ear notching tool that yields a 1cm a side notch
    2. Red Top blood tube: The preferred tube is a **VACUETTE® No Additive Tube:** 9ml.
    3. Shipping container rack that holds individual tubes in slots.
    4. Submission form: Click on the "Instructions for Excel Spreadsheet Template" and "Excel Spreadsheet for Electronic Submission".
    5. Disinfectant for rinsing notching tool: 10% bleach (eg.100ml (3oz) bleach in 900ml, (27oz) water).
    6. Clean rinse water: 3-5 gallon bucket. Change bucket water every 20-30 notches.
    7. Use disposable gloves and wear clean coveralls.
    8. Do not vaccinate or tattoo at the same time samples are taken.
2. Collection procedure
    1. Label Red Top collection tubes with the animal ID's and sequence number's (#1 through number in submission).
    2. Dip notching tool in disinfectant then ALWAYS rinse away disinfectant with copious quantities of clean water. **Caution:** Residual disinfectant on the notching tool will yield false negative results, therefore thorough rinsing with clean water is required.
    3. Take a proper size ear notch from a clean portion of the ear (see below). For comparison, a triangle notch size is superimposed on a nickel. Place notch into a labeled, dry, red top collection tube (no formalin, other liquid or separator gel; do not use snap-cap milk tubes or whirl-pak bags). **Caution:** Collected ear notch **MUST** be free of contaminating dirt, feces, tattoo ink or BVD vaccine.

Best location for taking ear notch.

Correct size of ear notch = ~1cm per side

3. Ear notch sample storage and shipping:
   Store collected ear notches for a maximum** of 72 hours at refrigerator temperatures and ship overnight to the WVDL on cold packs. This allows for a Friday collection and Monday shipping.

**Collection and storage of ear notches in clinics or on farms over the course of weeks or months causes sample degradation, making our testing invalid. Poor quality samples will be rejected.

SERUM SAMPLES

1. WVDL recommends serum separator tubes (aka tiger top tubes) over the non-additive red top blood tubes. Serum separator tubes help reduce hemolysis of samples and provide cleaner serum for testing.
2. Currently we supply serum separator tubes as a very low cost. They can be ordered here.

NASAL SWAB SAMPLES

1. WVDL recommends using BVD Nasal Swab Kit for large submission of BVDV PCR testing.
2. Currently we will supply the first kit free of charge for you to try out, please contact us to request the kit. They can also be ordered here.

	Recommended Test	Could also use:	Recommended sample type	Inappropriate sample type
Transient /Acute Infection (detection of virus)	Individual PCR	Virus isolation *NOTE:Timing of collection during viremia stage will be critical for successful isolation*	Whole blood *NOTE: Detection of acute infection requires use of whole blood because this sample contains white blood cells.*	Ear notch Serum
Persistent Infection (detection of virus)	Pool PCR for screening herds Individual PCR	Virus isolation Antigen capture ELISA (ACE) *NOTE: May be subject to maternal derived antibody interference. ACE should only be used for regulatory testing.*	Nasal swabs Whole blood Ear notch Serum *(only suitable for animal >9 wks old)*	Serum in animals < 9 weeks due to potential immune clearance Ear notch contaminated with tattoo ink
Testing for Antibody	Serum Neutralization *NOTE: SN samples should be taken 2- 3 weeks apart for paired testing.*		Serum	

Pathology & Professional Veterinary Services

Necropsy & Pathology testing fee changes

Necropsy and pathology service fee changes are effective on July 1, 2021. See the new Pathology Price Sheet on our website at http://www.wvdl.wisc.edu/.

The changes in fees reflect the rising costs of maintaining high quality pathology and testing services. Necropsy services are essential and remain a great value to Wisconsin animal owners. The new necropsy and pathology fees went into effect on July 1, 2021 and will be reflected on August invoices.

We appreciate the opportunity to provide these necropsy and pathology services as well as your ability to adjust along with us. We remain committed to serving you, your business and the animal health profession with accurate, reliable results and exceptional customer service.

If there are any questions, comments or concerns regarding these fee changes please contact the WVDL via email at info@wvdl.wisc.edu or via phone at 608.262.5432.

Bacteriology

Quantification of *Salmonella enterica* subspecies *enterica* Serotypes/Serogroups Identified at the WVDL from 2019 to 2020

Below are the serotypes and serogroups of *Salmonella* identified at the WVDL in 2019 and 2020. *Salmonella enterica* subspecies *enterica* contains greater than 2,500 different serotypes, also called serovars. Each serotype is identified at the WVDL using various sera that bind and agglutinate if a particular molecular pattern is present on the surface of the *Salmonella* bacterium in question. These agglutination reactions are run and positive reactions are used to first group (Group A, B, C1, C2, D1, E1, and G) the *Salmonella* isolate and then serotype it to identify a specific serotype name such as *Salmonella* ser. Heidelberg is in the Group B along with *Salmonella* ser. Typhimurium.

At the WVDL, not all *Salmonella* are serotyped fully as to save money for our clients. Often for bovine cases, only one isolate from an animal or sample will be fully serotyped and the other *Salmonella* isolates will be serogrouped. As long as the other *Salmonella* isolates serogroup (e.g. serogroup B for *Salmonella* ser. Heidelberg) to the same serogroup as the fully serotyped *Salmonella* within the same accession, then those *Salmonella* are only serogrouped not serotyped.

As summarized in **Table 1 (2019) and Table 2 (2020)** below and for reasons mentioned above, more serogroups have been identified than serotypes as a single case may have many *Salmonella* serogrouped, but only one *Salmonella* isolate that was serotyped. As an example, a group of 10 feces submitted for *Salmonella* culture might obtain 5 *Salmonella* isolates (5 of the 10 animals had *Salmonella* isolated), where only one will be serotyped and the other four will be serogrouped (e.g. 1 serotype reported and 4 serogroups reported).

The WVDL identified a total of 1,114 *Salmonella* isolates in 2019 and 1,529 in 2020. Of those isolates

42.5-57.0% were bovine, 39-45.8% were avian, 0.4 – 0.6% were equine and 6.5-11.1% were canine, porcine, caprine, and feline in origin. The tables below do not include isolates that could not be fully serotyped by WVDL or the National Veterinary Services Laboratories (NVSL) and do not include monophasic *Salmonella*, with few exceptions. Interestingly, we've seen an increase in *Salmonella* isolated from canine, porcine, caprine and feline (other) from 1% in 2018 to 11.1% in 2020.

TABLE 1: All *Salmonella enterica* subspecies *enterica* serotypes/serogroups identified in 2019.

2019 *Salmonella* Serotypes Isolated	All species	Bovine	Avian	Equine	Other
	1114	640	437	7	72
Group B	12	12	0	0	0
SALMONELLA AGONA	22	8	6	2	6
SALMONELLA BRANDENBURG	4	4	0	0	0
SALMONELLA DERBY	1	0	0	0	1
SALMONELLA HEIDELBERG	10	10	0	0	0
SALMONELLA KAAPSTAD	2	2	0	0	0
SALMONELLA READING	2	0	2	0	0
SALMONELLA SAINTPAUL	2	0	0	0	2
SALMONELLA SCHWARZENGRUND	18	11	6	0	1
SALMONELLA TYPHIMURIUM	76	41	22	3	10
Total	**149**	**88**	**36**	**5**	**20**
Group C1	2	1	0	1	0
SALMONELLA BAREILLY	8	0	0	0	8
SALMONELLA BRAENDERUP	3	1	0	1	1
SALMONELLA INFANTIS	21	4	15	0	2
SALMONELLA LIVINGSTONE	2	2	2	0	0
SALMONELLA MBANDAKA	16	10	4	0	2
SALMONELLA MONTEVIDEO	103	94	4	0	5
SALMONELLA NORTON	1	1	0	0	0
SALMONELLA NORWICH	2	2	0	0	0
SALMONELLA OHIO	1	0	1	0	0
SALMONELLA OTHMARSCHEN	4	4	0	0	0
SALMONELLA THOMPSON	1	0	1	0	0
Total	**164**	**119**	**27**	**2**	**18**
Group C2-C3	1	1	0	0	0
SALMONELLA ALBANY	2	0	2	0	0
SALMONELLA BOVISMORBIFICANS	4	3	0	0	1
SALMONELLA HADAR	6	0	6	0	0
SALMONELLA HERSTON	1	0	1	0	0
SALMONELLA KENTUCKY	165	5	160	0	0
SALMONELLA MANHATTAN	1	1	0	0	0
SALMONELLA MUENCHEN	8	7	0	0	1
SALMONELLA NEWPORT	27	26	0	0	1
Total	**215**	**43**	**169**	**0**	**3**
Group D	10	9	0	0	1
SALMONELLA DUBLIN	142	141	0	0	1
SALMONELLA ENTERITIDIS	8	1	3	0	4
SALMONELLA OUAKAM	4	3	1	0	0
SALMONELLA PANAMA	17	8	9	0	0

Total	**181**	**162**	**13**	**0**	**6**
Group E	6	4	0	0	2
SALMONELLA ANATUM	30	25	7	0	3
SALMONELLA GIVE	15	12	0	0	3
SALMONELLA LIVERPOOL	2	0	2	0	0
SALMONELLA MELEAGRIDIS	11	10	0	0	1
SALMONELLA MUENSTER	14	8	5	0	1
SALMONELLA SENFTENBERG	59	2	57	0	0
SALMONELLA UGANDA	35	5	30	0	0
Total	**172**	**66**	**101**	**0**	**10**
Group G	0	0	0	0	0
SALMONELLA AGBENI	1	1	0	0	0
SALMONELLA CUBANA	6	0	6	0	0
SALMONELLA HAVANA	68	1	67	0	0
SALMONELLA WORTHINGTON	1	0	0	0	1
Total	**76**	**2**	**73**	**0**	**1**
Group I	0	0	0	0	0
SALMONELLA BARRANQUILLA	4	0	4	0	0
Total	**4**	**0**	**4**	**0**	**0**
Group K	0	0	0	0	0
SALMONELLA CERRO	147	136	2	0	9
Total	**147**	**136**	**2**	**0**	**9**
Group M	0	0	0	0	0
SALMONELLA POMONA	1	0	1	0	0
Total	**1**	**0**	**1**	**0**	**0**
Group N	0	0	0	0	0
SALMONELLA SOEGRENGA	2	2	0	0	0
Total	**2**	**2**	**0**	**0**	**0**
Group O	2	2	0	0	0
SALMONELLA ALACHUA	1	0	1	0	0
Total	**3**	**2**	**1**	**0**	**0**

TABLE 2: All *Salmonella enterica* subspecies *enterica* serotypes/serogroups identified in 2020.

2020 *Salmonella* Serotypes Isolated	All Species	Bovine	Avian	Equine	Other(1)
	1529	650	701	6	170
SALMONELLA ARIZONAE/DIARIZONAE	1	0	0	0	1
Group B*	9	7	0	0	2
SALMONELLA AGONA	31	10	1	3	17
SALMONELLA SCHWARZENGRUND	11	3	4	0	4
SALMONELLA TYPHIMURIUM	120	56	35	1	28
SALMONELLA MONOPHASIC TYPHIMURIUM(2)	10	8	0	0	2
SALMONELLA HEIDELBERG	4	4	0	0	0

SALMONELLA BRANDENBURG	10	9	1	0	0
SALMONELLA SAINTPAUL	7	1	0	0	6
SALMONELLA BUDAPEST	2	0	0	0	2
SALMONELLA DERBY	1	0	0	0	1
SALMONELLA KIAMBU	6	3	0	0	3
Total	**212**	**101**	**41**	**4**	**66**
Group C1*	1	1	0	0	0
SALMONELLA INFANTIS	68	5	62	0	1
SALMONELLA TENNESSEE	1	0	1	0	0
SALMONELLA BRAENDERUP	1	1	0	0	0
SALMONELLA OTHMARSCHEN	4	4	0	0	0
SALMONELLA MONTEVIDEO	102	92	4	0	6
SALMONELLA THOMPSON	10	2	8	0	0
SALMONELLA HARTFORD	2	1	1	0	0
SALMONELLA ORANIENBURG	9	0	0	0	9
SALMONELLA RISSEN	1	0	1	0	0
SALMONELLA MBANDAKA	10	3	4	0	3
SALMONELLA LUBBOCK	2	2	0	0	0
Total	**211**	**111**	**81**	**0**	**19**
Group C2-C3*	0	0	0	0	0
SALMONELLA ALTONA	4	1	1	0	2
SALMONELLA KENTUCKY	326	8	315	0	3
SALMONELLA LITCHFIELD	1	0	0	0	1
SALMONELLA NEWPORT	41	16	6	0	20
SALMONELLA MUENCHEN	28	3	22	0	3
SALMONELLA BOVIS MORBIFICANS	5	3	0	1	1
Total	**405**	**31**	**343**	**1**	**30**
Group D*	4	4	0	0	0
SALMONELLA PANAMA	9	5	4	0	0
SALMONELLA DUBLIN	137	134	0	0	3
SALMONELLA ENTERITIDIS	2	0	1	0	1
SALMONELLA OUAKAM	2	0	2	0	0
Total	**154**	**143**	**7**	**0**	**4**
Group E*	0	0	0	0	0
SALMONELLA MELEAGRIDIS	12	9	2	0	1
SALMONELLA MUENSTER	36	13	5	0	17
SALMONELLA SENFTENBERG	180	4	175	0	1
SALMONELLA UGANDA	5	1	0	0	4
SALMONELLA GIVE	35	20	5	0	10
SALMONELLA ANATUM	15	13	0	1	1
SALMONELLA ORION	3	1	1	0	1
SALMONELLA KOUKA	1	1	0	0	0
SALMONELLA LIVERPOOL	8	3	5	0	0
Total	**295**	**65**	**193**	**1**	**35**
Group G*	1	0	0	0	0

SALMONELLA CUBANA	4	2	1	0	1
SALMONELLA HAVANA	80	79	0	0	1
SALMONELLA IDIKAN	1	0	0	0	1
SALMONELLA KEDOUGO	1	1	0	0	0
SALMONELLA WORTHINGTON	4	3	0	0	1
Total	**91**	**85**	**1**	**0**	**4**
Group I	0	0	0	0	0
SALMONELLA BARRANQILLA	1	1	0	0	0
Total	**1**	**1**	**0**	**0**	**0**
Group K*	0	0	0	0	0
SALMONELLA CERRO	122	111	2	0	9
Total	**122**	**111**	**2**	**0**	**9**
Group O*	13	2	9	0	2
SALMONELLA ALACHUA	19	0	18	0	1
Total	**32**	**2**	**27**	**0**	**3**
Group R	0	0	0	0	0
SALMONELLA JOHANNESBERG	6	0	6	0	0
Total	**6**	**0**	**6**	**0**	**0**

*Refers to *Salmonella* that was grouped but not serotyped.
(1) Other Includes Canine, Porcine, Caprine, and Feline.
(2) *Salmonella* 4,[5],12:i:- is believed to be the monophasic variant of *Salmonella* ser. Typhimurium. *Monophasic Salmonella* ser. Typhimurium cannot be confirmed at the WVDL due to a lack of sera needed to perform confirmation.

Changes to Antimicrobial Susceptibility Testing Starting in September

In an effort to participate further in Antimicrobial Stewardship, the WVDL will be changing how we report antimicrobial susceptibility testing (AST). Antimicrobial Stewardship at the diagnostic laboratory level includes, "positively affecting clinical outcomes, help maintain antimicrobial effectiveness, assist clinicians in using antimicrobial agents safely, and minimizing the selection of resistant pathogens, laboratories must use a standardized, well-defined method for performing AST."(1)

The WVDL uses primarily the broth microdilution method, which quantitatively measures the *in vitro* activity of an antimicrobial agent against a particular bacterial pathogen. Antimicrobial agents, in a serial dilution, are prepared and mixed with a standardized suspension of the bacterium. The WVDL does not prepare these drug dilutions in-house, but rather relies on panels provided in 96-well plate format by TREK Diagnostics (ThermoFisher Scientific). These are incubated and the minimum inhibitory concentration (MIC), which is the highest dilution (lowest concentration) of an antimicrobial drug that completely inhibits bacterial growth, is determined. Based on the MIC, the resistance, intermediate response or susceptibility of an organism, from a particular host species and tissue to a particular antimicrobial is established using the Clinical and Laboratory Standards Institute (CLSI) breakpoints.

A breakpoint is established by CLSI utilizing microbiological characteristics, pharmacokinetic-pharmacodynamic (PK/PD) parameters, and/or clinical outcome data. Veterinary-specific breakpoints were established with particular attention to the product label. The MIC for the particular pathogen-drug combination is used against the CLSI breakpoint established for that pathogen-drug combination to determine interpretative criteria which is susceptible, intermediate or resistant (see example).

Example: *Escherichia coli* isolated from a canine urine sample. The MIC for enrofloxacin was 0.25 µg/mL. Using the breakpoints listed below, the MIC for the isolate is categorized as susceptible because it is < 0.5 µg/mL.(1)

Interpretive Category	**Enrofloxacin Breakpoints (MIC, µg/mL)**
Susceptible	≤ 0.5
Intermediate	1 – 2
Resistant	≥ 4

The CLSI guidelines are specific to a particular bacterium isolated from a particular host species' tissue. As an example, there are specific breakpoints for particular antimicrobial agents that have been established for bovine respiratory disease pathogens such as *Pasteurella multocida*, *Mannheimia haemolytica* and *Histophilus somni*. These breakpoints do not apply to these bacteria isolated from non-respiratory tissues from cattle.

Additionally, these breakpoints do not apply to non-bovid species such that a *P. multocida* isolated from a cat would not get these same breakpoints. Therefore, the CLSI guidelines utilize a grouping system for interpretations of antimicrobial agents and their uses for veterinary pathogens.

- **Group A:** includes antimicrobial agents with VETERINARY-SPECIFIC breakpoints and interpretive categories that are considered appropriate for routine, primary testing for food and companion animals. These antimicrobial agents are considered first to report and use, and are preferred over using those with human medical breakpoints. These Group A compounds have demonstrated an acceptable level of correlation between *in vitro* susceptibility test results and clinical outcome.
- **Group B:** includes antimicrobial agents with veterinary-specific breakpoints and interpretive categories but are considered antimicrobials that should only be tested and reported as 'drugs of last resort'. The Subcommittee on Veterinary Antimicrobial Susceptibility Testing (VAST) considers these antimicrobials to be 'drugs of last resort' and concern exists for selecting for resistance, which could be transferred from animals to humans. The veterinary laboratory can report these at their discretion but are mostly used as antimicrobial resistance monitoring.
- **Group C:** includes antimicrobial agents that use HUMAN medical breakpoints and interpretive categories. These agents may perform adequately, but outcomes for many veterinary applications have not been demonstrated. The veterinary laboratory can report these at their discretion.
- **Group D:** include antimicrobial agents that are regulatory agency-approved for use in the specific animal species. Although quality control data is available, these antimicrobial agents DO NOT have CLSI-approved veterinary-specific or human medical breakpoints or interpretive categories. These agents may be approved for use in other animal species and have veterinary-specific breakpoints in those animals. However, it is not recommended to use breakpoints set for a particular animal species to be applied to a different animal species. This is because there are differences in dosages and pharmacokinetics between animals, people and between animal species. Thus, these agents should be reported selectively before extra-label use agents (Group D), but after agents in Group B.
- **Group E:** includes antimicrobial agents that are NOT APPROVED but may be used in an extra-label manner per the Animal Medicinal Drug Use Clarification Act of 1994 (AMDUCA) guidelines in the US. These agents may be selectively tested and reported and are often used for antimicrobial resistance monitoring. Group E may also include certain antimicrobial agents that are used only for a specific infection site (such as nitrofurantoin for treating urinary tract infections) in non-food-producing animals.(1)

TABLE 3: Antimicrobial Agents that could be Considered for Routine Testing by Veterinary Microbiology Laboratories.

	Animal Species						
Test/Report Group	Swine	Cattle	Bovine Mastitis	Poultry	Horses	Dogs	Cats
Group A- Vet-specific breakpoints					Amikacin	Amikacin	
						Amoxicillin-clavulanate	Amoxicillin-clavulanate
	Ampicillin	Ampicillin			Ampicillin	Ampicillin	Ampicillin
					Cefazolin	Cefazolin	
						Cefovecin	Cefovecin
			Cefoperazone				
	Ceftiofur	Ceftiofur	Ceftiofur		Ceftiofur		
						Cephalexin	
						Cephalothin	
						Clindamycin	
		Danofloxacin					
						Difloxacin	
					Doxycycline	Doxycycline	
	Enrofloxacin	Enrofloxacin			Enrofloxacin	Enrofloxacin	Enrofloxacin
	Florfenicol	Florfenicol					
		Gamithro-mycin					
					Gentamicin	Gentamicin	
						Marbo-floxacin	Marbo-floxacin
					Minocycline	Minocycline	
						Orbifloxacin	Orbifloxacin
	Penicillin G	Penicillin G			Penicillin G		
			Penicillin-novobiocin				
			Pirlimycin				
						Pradofloxacin	Pradofloxacin
		Spectino-mycin					
	Tetracycline	Tetracycline				Tetracycline	
	Tiamulin						
	Tildipirosin	Tildipirosin					
	Tilmicosin	Tilmicosin					
	Tulathro-mycin	Tulathro-mycin					

Group B- vet-specific breakpoints; drugs of last resort						Ceftazidime	
				Enrofloxacin			
						Levofloxacin	
						Piperacillin-tazobactam	
Group C- human breakpoints							Amikacin
			Ampicillin				
					Azithromycin	Azithromycin	
							Cefazolin
							Cephalexin
					Chloram-phenicol	Chloram-phenicol	Chloram-phenicol
	Clindamycin						Clindamycin
						Colistin	
							Doxycycline
	Erythromycin	Erythromycin	Erythromycin	Erythromycin	Erythromycin	Erythromycin	Erythromycin
	Gentamicin			Gentamicin			Gentamicin
							Minocycline
						Nitrofurantoin	
	Oxacillin	Oxacillin	Oxacillin	Oxacillin	Oxacillin	Oxacillin	Oxacillin
			Penicillin	Penicillin		Penicillin	Penicillin
					Rifampin	Rifampin	Rifampin
				Spectino-mycin			
	Sulfonamides	Sulfonamides		Sulfonamides		Sulfonamides	Sulfonamides
			Tetracycline	Tetracycline	Tetracycline		
						Tobramycin	Tobramycin
				TMS	TMS	TMS	TMS
Group D- Only QC ranges available (breakpoints not established)	Apramycin						
				Ceftiofur		Ceftiofur	
	Cefquinome	Cefquinome	Cefquinome		Cefquinome		
				Clindamycin			
	Gamithro-mycin						
			Kanamycin-cephalexin				
	Spectino-mycin						
	Tylosin	Tylosin					
Group E- drugs that may be tested and selectively reported if isolate is resistant to Group A, B or C agents	Amikacin	Amikacin					
		Gentamicin					
					Imipenem	Imipenem	Imipenem
						Linezolid	Linezolid
					Meropenem	Meropenem	Meropenem
	TMS	TMS					
	Tylvalosin			Tylvalosin			
					Vancomycin	Vancomycin	Vancomycin

Please see Vet01 Supplement for more information.

Currently, the WVDL provides interpretations based on CLSI guidelines as well as some breakpoints supplied by Trek Diagnostics (ThermoFisher Scientific). The breakpoints supplied by TREK Diagnostics will no longer be used starting on September 2, 2021. The WVDL will solely be using breakpoints supplied by CLSI and will be reporting per Table 3. Therefore, the WVDL will report mostly Group A and Group C antimicrobial agents based on the pathogen and what species and tissue in that host species the pathogen was isolated from. On occasion, some Group B, D and E antimicrobials may be interpreted with an MIC and interpretive criteria based on CLSI Vet01(1) and Vet09(2) guidelines. An example is applying *M. haemolytica* breakpoints for bovine respiratory disease to other members of the *Pasteurellaceae* family is acceptable(2). As well, the CLSI Vet09 extrapolates the *Staphylococcus aureus* breakpoints and interpretive criteria for bovine mastitis so that Gram-positive cocci (but not *Enterococcus*) can be interpreted.(2)

There will be fewer antimicrobials reported with interpreted categories as we move to reporting only CLSI approved on September 2, 2021.

Please contact us for more information regarding AST or if additional antimicrobial agent breakpoints are needed.

(1)CLSI Performance Standards for Antimicrobial Disk and Dilution Susceptibility tests for Bacteria Isolate from Animals. CLSI, Vet01, Edition 5.
(2)Understanding Susceptibility test Data as a Component of Antimicrobial Stewardship in Veterinary Setting.

CLSI, Vet09, Edition 1.

www.wvdl.wisc.edu

Our mailing address is:
445 Easterday Lane
Madison, WI 53706
info@wvdl.wisc.edu