Welcome to the MU Veterinary Medical Diagnostic Laboratory summer 2019 newsletter. I hope your summer is off to a great start! I have several items of news that I would like to share.

To comply with the new USDA policy, the MU VMDL will not conduct active surveillance on African swine fever (ASF), classical swine fever (CSF) and Pseudorabies (PRV). The VMDL will continue to test ASF and CSF for foreign animal disease (FAD) investigation and perform PRV testing for movement.

If you see swine cases resembling ASF/CSF, such as high fever, depression, anorexia, hemorrhages, abortion, vomiting, diarrhea and increased mortality, please contact the Missouri state veterinarian who will send field veterinarians to investigate the case and collect samples for ASF/CSF testing. The VMDL will conduct the ASF/CSF PCRs as soon as samples arrive in the lab.

The VMDL is also approved to conduct passive ASF/CSF FAD investigations. Whenever appropriate, VMDL pathologists will collect tissue samples from swine necropsy cases for ASF/CSF testing. In all FAD investigations, VMDL results are considered preliminary and will be finalized after USDA FADDL results become available.

If you are interested in the trends of food animal diseases diagnosed by the lab, please explore the VMDL website. We encourage you to contact us with any issues, suggestions, or comments about the website and portal.

To support livestock and poultry producers, the VMDL will offer volume discounts on many lab tests, especially ELISA and PCRs. Our goal is to provide high quality and affordable service to all practicing veterinarians, owners, and producers. Due to increasing costs of reagents and supplies, we will slightly increase (2 to 3 percent) our fees for companion animal testing. The new fee guide was published on July 1, 2019.

As always, your feedback is important to us because the VMDL is here to serve all of you and the state of Missouri.

Best regards,
Shuping Zhang, Director, Veterinary Medical Diagnostic Laboratory
Professor, Veterinary Pathobiology
Biopsy Submission Reminders
Our biopsy service continues to be extremely popular — thank you for your support! The biopsy fee includes up to three special stains/IHC, as well as multiple sites from the same patient at no additional charge. To help us provide high quality and timely reports, please remember the following when submitting your samples:

- Include an appropriate history (at minimum, number of submitted tissues and location)
- Incise larger tissues to allow proper fixation (sections should be less than one-quarter-inch thick)
- Submit in non-breakable plastic containers with tight screw-on lids
- Use an adequate amount of 10 percent neutral buffered formalin (the ideal ratio is 20 parts formalin to one part tissue)

Pre-filled formalin containers are available for purchase through the VMDL. Download and complete the order form to have these supplies shipped directly to your clinic.

CWD Testing in Captive Cervids
We often receive questions about CWD testing in farmed/captive deer and other cervids. While state conservation departments manage surveillance testing in wild populations, the USDA manages the testing and interstate movement of captive cervids. Therefore, the testing requirements and lab certifications differ slightly.

The available tests are the same for both wild and captive deer, and involve an ELISA screening test followed by IHC for confirmation of suspect positives. The VMDL can run both tests for wild deer; however, all IHC confirmation for captive deer must be performed at NVSL. Therefore, please remember to submit fresh tissues for ELISA testing at the VMDL. If only formalin-fixed tissues are submitted for captive cervids, they must be forwarded on to NVSL, which will result in longer turnaround times.

If you have any questions about sample collection or submission requirements, please contact us.

Buffalo Gnats Threaten Livestock and Poultry
With widespread flooding across Missouri, conditions are favorable for the buffalo gnat this year. Keep an eye out for these biting flies, also known as black flies, especially if you live near a waterway.

Buffalo gnat eggs are laid in cool, running water where the larvae hatch and mature. The larvae are sensitive to environmental factors such as oxygen levels, sediment, and water temperature. When the water temperature rises above 75 degrees, the larvae cannot survive. Therefore, the season for these insects is generally over in the early summer.

Buffalo gnats feed on blood from humans and livestock in order to reproduce. Large numbers of bites can cause livestock deaths due to blood loss or anaphylactic shock. Additional risks to poultry include suffocation when the insects are inhaled, and leukocytozoon infection. Buffalo gnats are daytime feeders, so keep your livestock indoors with fans or other cooling devices during the day, if possible. Permethrin-based products can also be used to protect your animals. Stay vigilant and the season will be over soon.

Contagious Equine Metritis Testing
Horse breeders and equine veterinarians, the VMDL is one of only a few laboratories in the United States that is authorized to test equids for Contagious Equine Metritis (CEM). Caused by the bacterium *Taylorella equigenitalis*, CEM causes infertility and early embryonic loss in mares. It is a foreign animal disease in the United States, so testing is usually done only by breeders or veterinarians who are shipping semen, embryos or breeding horses overseas. If you or your clients are considering shipping breeding stock or reproductive products to countries that require CEM testing then we can provide that testing. The exact sites to be sampled and the timing of sampling vary depending on the...
CEM, continued

country you are shipping to, so contact a regulatory veterinarian in the target country or contact your USDA veterinarian for guidance.

Once you have determined the sites to be sampled and timing of your samples, give us a call before you actually take your swabs. The specialized media we use for Taylorella testing must be ordered weeks in advance and we do not routinely keep it in stock. If we do not know the samples are coming, we may be unable to test them. We can also provide the charcoal Amies media that is required for CEM testing as well as mini-tip swabs for sampling mares.

Regulatory requirements for sampling and shipping of CEM swabs are stringent, so reach out to your regulatory veterinarian before beginning the sampling process. The culture itself takes a full week once we receive the swabs. If you have any questions about the culture process or need media, call the Bacteriology section at 573-884-9245.

Which Leptospirosis Test Should I Use?
The VMDL offers two different testing methodologies for canine leptospirosis: a qPCR test, which detects the nucleic acid of pathogenic leptospires, and a six serovar microscopic agglutination test (MAT), which detects antibodies. It is important to understand testing benefits and limitations when deciding which test(s) to use for your patient.

The MAT is the most commonly used diagnostic test for canine leptospirosis. It’s best to collect two samples about two weeks apart when using the MAT. A rising titer is suggestive of a recent infection, since titers due to past vaccination or chronic infection are unlikely to increase significantly during that interval. While a four-fold increase is what we traditionally look for, the titer increase may be affected by the initiation of antimicrobial therapy. A single titer can be difficult to interpret, since dogs are often seronegative early in the course of disease, and vaccination has been shown to induce persistently high titers in some cases. Cross-reactivity between non-vaccinal serovars can further confuse things. However, when used properly, the MAT is a useful diagnostic tool.

The PCR test can readily detect the nucleic acid of pathogenic leptospires in urine and whole blood samples. However, it is best used early in the course of disease prior to treatment with antimicrobials. Recent use of antimicrobials can cause false negative PCR results. The number of organisms is highest in the blood for the first 10 days of infection, and subsequently is highest in the urine of infected animals. If the duration of infection is unclear, submission of both blood and urine is recommended.

Urine PCR testing may also detect chronic infections, provided the animal has not recently been treated with antimicrobials. Apparently healthy dogs may also shed leptospires in their urine, so a positive urine PCR test should be interpreted along with relevant clinical signs.

Contact us if you have questions about test selection for canine leptospirosis. We’re happy to help.

Pinkeye Season Is Here
Cattle producers will want to start keeping an eye out (pun intended!) for pinkeye in their cattle. Pinkeye, also known as infectious bovine keratoconjunctivitis, is most often caused by the bacteria Moraxella bovis and Moraxella bovoculi, although some mycoplasmal and viral infections can produce the same clinical signs. Clinical signs include squinting, red eye, runny eye, opacity of the cornea and ulcers of the cornea. In severe cases the cornea can rupture, which may lead to loss of vision or even require removal of the eye. Pinkeye may be spread by flies, so pinkeye control in a herd can be greatly assisted by appropriate fly control measures such as insecticide ear tags or back rubs. Vaccination of cattle may also help in heavily affected herds. In addition, cutting of grasses may reduce the chances of mechanical trauma to eyes. Trace mineral status might also be investigated, as either copper or selenium deficiency may contribute to an increased incidence of pinkeye.

Treatment of clinical pinkeye cases can be done with antibiotics, but producers may wish to confirm the presence of the causative bacteria before investing in antibiotic therapy. The VMDL can assist you in testing for pinkeye. For bacterial culture, submit a swab of the inside of the lower eyelid or of the ulcerated cornea in transport media contain-
Pinkeye, continued

ing charcoal or liquid Amies media. Swabs of ocular discharge on the animal’s face tend to be heavily contaminated and are not recommended. Shipping of dry swabs (without transport media) is also not recommended, as the organism is likely to die off during transport. If you need swabs or media, call the Bacteriology section at 573-884-9245 and we can send you some. We can also assist in saving of bacterial isolates for bacterin production, if desired.

Conjunctivitis in small ruminants can also occur during the summer months. However, the cattle _Moraxella_ species of bacteria are unlikely to be the cause. Sheep and goats more often get conjunctivitis from _Mycoplasma_ or _Chlamydia_ bacteria, although again viruses may also play a role. If you wish to test small ruminants for conjunctivitis, send a swab in liquid transport media to the VMDL for mycoplasma PCR. Treatment of mycoplasmal conjunctivitis with antibiotics may help resolve clinical signs, but it does not eliminate the bacterium and affected animals may develop a carrier state.

**Symposium on Bovine Anaplasmosis**

On May 20, two representatives of the VMDL, Lauren Delaney, DVM and Rosalie Ierardi, DVM, attended the Second Symposium on Bovine Anaplasmosis at Kansas State University in Manhattan, Kansas. The conference was attended by veterinarians and producers and covered a range of topics including transmission, prevalence, and pathogenesis of this widespread tickborne disease of cattle.

_Anaplasma marginale_ is a rickettsial parasite of red blood cells that is spread primarily by _Dermacentor_ spp. ticks, which are amplifying hosts. It can also be spread mechanically by biting flies and any equipment contaminated with blood, most notably needles and castration or dehorning equipment.

Once a cow is infected with _A. marginale_, she enters an acute phase characterized by a rapid increase in numbers of the rickettsia in the blood, during which cow may show clinical signs such as fever, lethargy, icterus, exercise intolerance, abortions or death. Aggressive behavior has also been reported. This acute phase is the time during which the organism can be detected on a blood smear.

If the cow survives the acute phase, she enters a chronic phase during which numbers of rickettsia in the blood wax and wane every 10 to 14 days (cyclic rickettsemia). These cows are lifelong persistently infected carriers. Because blood levels of _A. marginale_ are very low, compared to the acute phase, the organism is generally undetectable on blood smears. For the same reason, mechanical transmission, e.g. by biting flies, is less efficient in these animals. However, ticks can still readily spread the disease, since _A. marginale_ multiples rapidly in the tick gut.

Since untreated infected cows remain subclinical carriers for life, a positive antibody titer is a reliable way to identify infected animals. Antibody testing by cELISA is a useful screening test available at the VMDL. PCR testing is also available if additional confirmation is desired.

From 2015 to 2017, the VMDL received a total of 1,413 specimens for _A. marginale_ cELISA testing. Female cattle accounted for the majority of specimens received, at 83 percent of the 1,252 specimens for which the animal’s sex was provided. Results are shown in Table 1. This data should be interpreted with caution, as diagnostic samples can be biased toward higher prevalence than would be observed in a randomly selected group of animals. In 2013, slaughterhouse surveillance yielded a 35 percent apparent seroprevalence in 54 Missouri-origin beef cattle. This data should likewise be interpreted with caution as the sample size is small.

**Table 1: MU VMDL A. marginale cELISA Results**

<table>
<thead>
<tr>
<th>Year</th>
<th>Positive</th>
<th>Total</th>
<th>Percent Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>2015</td>
<td>207</td>
<td>383</td>
<td>54%</td>
</tr>
<tr>
<td>2016</td>
<td>230</td>
<td>534</td>
<td>43%</td>
</tr>
<tr>
<td>2017</td>
<td>212</td>
<td>496</td>
<td>43%</td>
</tr>
<tr>
<td>Total Period</td>
<td>649</td>
<td>1413</td>
<td>46%</td>
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