

WVDL Winter Newsletter

January 2023



Wisconsin Veterinary Diagnostic Laboratory UNIVERSITY OF WISCONSIN-MADISON



Message from the Director

Happy New Year from our team at WVDL! We appreciate you taking the time to read our newsletter. As always, there is a wealth of information from our diagnostic sections that can inform you and your clients to sustain and improve animal health. We also want to make sure that we are facilitating sample submission to get the best diagnostic results possible.

WVDL has not been immune to the drastic increases in the costs of providing goods and services to all of our clients and stakeholders. The Consumer Price Index was 7% in 2021 and 6.5% in 2022. While we were able to keep our fee increase to 2% for FY23, WVDL's fee increase for FY24, starting July 1, 2023 will be 6%. This price

increase is to counter the 8-10% increases in supply and reagent costs, utilities, and personnel costs to maintain diagnostic and professional services. Depending on growth, we may be able to decrease this fee increase, like we did in 2022 when we decreased the fee increase from 3 to 2%.

Back to exciting news - mark your calendars for Thursday, December 7th for our **2023 Bovine Germplasm Export meeting at WVDL**. This will coincide with our table top exercise of the Bovine Germplasm Movement Plan in Madison on December 6th, which is currently being written after a year of work with state animal health officials, industry, and laboratory partners.

I would also like to direct your attention to the end of this newsletter for more information regarding FBI Agricultural Information shared to us by Special Agent Scott Mahloch - Weapons of Mass Destruction Coordinator of the Milwaukee FBI. Special agent Mahloch shared this information at our recent Annual Bovine Genetics meeting in December, 2022. These attached articles provide insightful information on the topics of agricultural economic espionage, biosecurity from a law enforcement perspective, and intellectual property protection.

Keep warm, stay safe, and we are looking forward to working with you in 2023!

Keith

In this Issue

- **Bacteriology Update**
- **Molecular Diagnostics Update**
- **Pathology Update**
- **Serology Update**
- **Virology Update**

Bacteriology

Please Send National Poultry Improvement Plan (NPIP) Testing Directly to the Barron Location

Whenever possible, please send NPIP testing directly to the Barron Laboratory. NPIP requires that environmental samples be setup for culture within 5 days from collection. It can be difficult to meet that

requirement if samples arrive late in the week to the Madison location, which does not setup cultures



for NPIP testing. Therefore, whenever possible, please send samples to the Barron Laboratory for the fastest turnaround time and to meet NPIP requirements. If additional samples are sent to the Barron location that need testing at the Madison location, the Barron staff are happy to route those samples to Madison once the NPIP samples have been removed and testing started. If you have any questions, please call, or email the WVDL Barron Laboratory.



Friday Drop Off Testing for Bacteriological Cultures

As a reminder, if you are planning to submit samples on a Friday afternoon for bacteriological culture, please do as early as possible. If samples arrive later in the day, they may not have time to be routed to bacteriology because they must be logged in, barcoded and reviewed prior to culture. The earlier samples are dropped off on Fridays, the more likely the sample will be cultured that afternoon. If you are submitting mastitic milk, please allow for thawing time or submit chilled, not frozen milk.

For the same reason, samples requiring a lot of preparation, such as the McMasters Quantitative Egg Count preferably need to be submitted in the morning.

Molecular Diagnostics & Virology

Highly Pathogenic Avian Influenza (HPAI): Preparing for Spring Sample Submissions

Wild birds can be infected with HPAI and show no signs of illness. They can carry the disease to new locations when migrating and are highly likely to pose a threat to the poultry industry across the nation in 2023. The Eurasian H5 strain HPAI outbreak that started in the Spring of 2022 is active in South America. While we have seen some relief in Wisconsin this winter, the outbreak is ongoing and animal health officials expect more cases from spring migration. All bird owners should review their biosecurity procedures and continue to stay vigilant to protect poultry and pet birds from HPAI.



Nationwide as of 1/23/2023, over 58.16 million birds have been affected from 310 commercial flocks and 428 backyard flocks. This is now considered the worst animal health emergency in the United States. The current HPAI information is available on the USDA website <https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/animal-disease-information/avian/avian-influenza/hpai-2022>. To date, WVDL has performed 4,432 HPAI PCR tests between March 2022 and December 2022. This has included 29 (11 commercial and 18 non-commercial) flocks across 18 Wisconsin counties which have been confirmed with HPAI for 2022. Additionally, HPAI has been identified in 9 wild bird cases and 5 mammalian cases in Wisconsin to date. As we head toward spring 2023, the WVDL is expecting to see an increase in avian influenza PCR testing again. Please follow and review the guidelines in the next article for preparedness in the upcoming spring migratory season.

What you need to know about Avian Influenza PCR testing at the WVDL

Media: WVDL is able to send media for AI testing upon request. The form and a video on proper sampling technique can be found at <https://www.wvdl.wisc.edu/forms/> under the "Supply Order Forms" header.

WVDL email contacts: One email address has been created to streamline the point of contacts at the WVDL. Please use AIsubmissions@wvdl.wisc.edu when contacting the WVDL about avian influenza questions and sample submissions.

WVDL submission forms: We have recently updated the avian submission form with boxes to check for which category of testing is requested. New forms can be found here: <https://www.wvdl.wisc.edu/forms/>

NOTE: This affects testing turnaround time, so please fill out the form completely and use the boxes provided.

Testing schedule: Routine testing is performed 2 days/week. The expected time for results to be reported is around 5-6 pm or later on testing day. Additional testing schedules will be added only when same day testing results are needed, thus advance arrangement is **required** for these types scenarios for expedited testing. Please reach out to us as soon as you anticipate urgent testing needs for permitted movement, control zone surveillance testing, restocking/re-movement testing, or over-mortality threshold testing. If there are any questions regarding additional information, please refer to resources available on the DATCP website:

- [DATCP avian influenza webpage](#) : This website includes a biosecurity self-assessment, prevention tips, and other information about the AI disease.
- [USDA Defend the Flock program](#) : This website provides biosecurity information and videos to learn about protecting your flock during disease outbreak situations and general flock health.
- [DATCP's AI resource center](#) : Fact sheets and brochures are available here to provide more information regarding food safety, protecting your pets, and biosecurity in relation to AI.

Any high increases in bird morbidity/mortality (or) abnormal clinical signs should be reported to DATCP at (800) 572-8981.

Pathology

WVDL Representation at the Annual American College of Veterinary Pathologists Meeting

Dr Andrea DuVall (WVDL microbiologist) and Dr Kelsey Brown (UW-Madison anatomic pathology resident) represented WVDL at the annual meeting of the American College of Veterinary Pathologists in Boston, MA, Nov 12-15 2022 with two poster presentations. Excellent work Drs. DuVall and Brown!

Severe Ulcerative Enteritis Associated with Copper Deficiency in an American Bison

Andrea L DuVall, Ryan M Breuer, Philip N Bochsler and Lorelei L Clarke
Wisconsin Veterinary Diagnostic Laboratory, Madison, WI

Introduction

- Bison herd (50 head) managed in NE Wisconsin translocated 2 yrs prior to herd investigation
- Shortly after translocation several animals were exhibited ill-thrift, wasting, diarrhea and death; overall mortality = 26.50
- Previous submissions indicated copper deficiency, herd was given free-choice mineral supplement for the last 6 mths
- 2-year-old male submitted to the WVDL for necropsy in December 2021

Infectious Disease Testing

Trappesella pyogenes was isolated from intestinal ulcers and lymph node

Other testing performed without detection:

- Salmonella* sp. (PCR and culture)
- Bovine viral diarrhea virus PCR
- Epizootic hemorrhagic disease virus PCR
- Bluetongue virus PCR
- Malignant catarrhal fever PCR
- Mycobacterium avium* ssp. *Paratuberculosis* PCR
- Bovine coronavirus PCR

Hepatic Trace Mineral Panel

Element	Concentration	Reference Range
Copper	0.02	0.01 - 0.04 ppm
Iron	98	50 - 150 ppm
Zinc	0.02	0.01 - 0.04 ppm
Manganese	0.02	0.01 - 0.04 ppm
Selenium	0.02	0.01 - 0.04 ppm
Cadmium	0.02	0.01 - 0.04 ppm
Cobalt	0.02	0.01 - 0.04 ppm
Chromium	0.02	0.01 - 0.04 ppm
Vanadium	0.02	0.01 - 0.04 ppm
Molybdenum	0.02	0.01 - 0.04 ppm
Strontium	0.02	0.01 - 0.04 ppm
Barium	0.02	0.01 - 0.04 ppm
Lead	0.02	0.01 - 0.04 ppm
Mercury	0.02	0.01 - 0.04 ppm
Antimony	0.02	0.01 - 0.04 ppm
Thallium	0.02	0.01 - 0.04 ppm
Bismuth	0.02	0.01 - 0.04 ppm
Fluorine	0.02	0.01 - 0.04 ppm
Boron	0.02	0.01 - 0.04 ppm
Silicon	0.02	0.01 - 0.04 ppm
Aluminum	0.02	0.01 - 0.04 ppm
Chlorine	0.02	0.01 - 0.04 ppm
Sulfur	0.02	0.01 - 0.04 ppm
Phosphorus	0.02	0.01 - 0.04 ppm
Oxygen	0.02	0.01 - 0.04 ppm
Nitrogen	0.02	0.01 - 0.04 ppm
Carbon	0.02	0.01 - 0.04 ppm

Proposed Pathogenesis

Copper Deficiency associated with:

- Reduced lysyl oxidase activity
- Reduced superoxide dismutase activity

Leads to:

- Reduced cross-linking of collagen and elastin in blood vessels
- Inability to counteract oxidative injury

Weaker enteric vessel walls/wear and tear damage = **inflammation, thrombin deposition** = thrombus formation in vessels

Thrombi lead to **infarction** and increased damage from **oxidative injury** = necrosis, ulceration and fibrinous adhesion formation

Case Follow-up

- T. pyogenes* is an unlikely primary cause for intestinal ulcers, but may have contributed to lesion severity
- No other infectious entities identified
- This bison had several lesions classically consistent with chronic copper deficiency (achromotrichia, poor body condition, diarrhea)
- Selenium deficiency may have contributed to myocardial fibrosis
- After this investigation, copper was supplemented as a high Cu-containing porcine grain ration and top-dressed hay with CuSO₄
- Remaining bison in the herd (24) reported as healthy and gaining weight year later after investigation

Figure 1: Gross necropsy findings from a 2yo M Bison

A: Posterior view of the dorsum (asterisk) showing poor body condition and faded hair coat
 B: Loops of distal jejunum with fibrin adhesions along serosal surfaces (arrow)
 C: The mucosal surface of distal jejunum disrupted by well-demarcated ulcers (arrow) with adjacent hemorrhage; similar ulcers also present in cecum and abomasum
 D: Coalescing areas of pallor and hemorrhage (arrow) efface the epicardium and myocardium

Figure 2: Histologic findings in grossly evident intestinal ulcers (A-C) and heart (D)

A: Distal jejunum, complete ulceration of the mucosa, exposing the submucosa, with necrosis, inflammation, congestion and bacteria extending through the muscularis layer; the serosa is markedly expanded by fibrin and inflammation - H&E, 40x
 B: Distal jejunum, gram negative and gram positive bacteria evident on the luminal surface extending into the ulcerated tissue - B&H, 200x
 C: Distal jejunum, thrombi present in vessels (arrow) with fibrinoid necrosis of vessel walls - H&E, 600x
 D: Heart, multifocal to coalescing loss of cardiomyocytes, replaced by extensive fibrosis - H&E, 40x (Inset: Heart, Gomori's Trichrome Stain, 200x)

Acknowledgements:

Special thanks to Dr. Scott McKay and Dr. Donald Socken for their contributions, as well as Torti Smith, HIASPCM and the pathology sciences staff at the WVDL for their aid in histopathology slide preparation and assistance on this case.

References:

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Cutaneous Histiocytosis in an Eastern Gray Squirrel (*Sciurus carolinensis*)

Kelsey Brown¹, Lorelei Clarke², Christoph Mans¹
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Introduction

- Reactive histiocytosis comprise non-neoplastic, proliferative histiocytic diseases for which the etiopathogenesis is poorly understood, though may be associated with immune dysregulation.
- This disease category can involve a single or multiple organs and demonstrate benign or malignant behavior.
- Reactive histiocytic disease are well-described in dogs and humans, less so in cats, and rarely in other species.

Case Report

A free-ranging adult, male Eastern gray squirrel (*Sciurus carolinensis*) from Wisconsin presented with bilateral, 13 mm-diameter ulcerated cutaneous masses at the base of the pinnae (Fig. 1), one of which was surgically excised.

Histopathology Results

- The mass comprised a dense population of pleomorphic round cells with histiocytic morphology infiltrating the dermis (Fig. 2-3).
- Few multinucleated giant cells (MNGCs, Fig. 4) and large numbers of lymphocytes and plasma cells interspersed with the histiocytic population.
- Anisocytosis and anisokaryosis were moderate and there was 1 mitotic figure per ten high-powered fields (2.37 mm²).
- The ulcerated epidermis was covered by a serocellular crust with clusters of Gram-positive cocci (interpreted as secondary colonization).
- No other infectious organisms were seen with HE, PAS, ZN, B&H, GMS, and Steiner stains.
- The round cells and MNGCs demonstrated strong cytoplasmic reactivity for Iba-1 (Fig. 5), scattered reactivity for MAC387, MHC II, and CD18, and no reactivity for CD79a, CD3, or E-cadherin.

Diagnosis

Cutaneous histiocytosis

Follow-up & Discussion

Following the biopsy, the contralateral mass spontaneously regressed, and the squirrel was released.

- This is the first report of a proliferative histiocytic disease in an Eastern gray squirrel. The lesion most closely resembles cutaneous histiocytosis of dendritic cell origin based on the cell morphology and IHC profile (Iba-1 positive, E-cadherin & MAC387 negative).
- Cutaneous histiocytosis has been reported in 4 European red squirrels (*S. vulgaris*) on postmortem examination, although the red squirrel population demonstrated visceral involvement and MHC II immunopositivity, with similar immunonegativity for CD18, MAC387, and E-cadherin.¹
- This condition should be considered as a differential for nodular and ulcerative skin lesions in squirrels, especially due to its macroscopic resemblance to multiple infectious diseases, most significantly squirrel fibroma virus (leporipoxvirus).

References

1. Smith SH, Stevenson K, Del Pozo J, Moss S, Meredith A. Atypical Histiocytosis in Red Squirrels (*Sciurus vulgaris*). *J Comp Pathol*. 2017; 130(4):446-450.

Acknowledgements

Thanks to the Histology Laboratory at the University of Georgia College of Veterinary Medicine for performing the immunohistochemistry and the care team at the Wisconsin Humane Society's Wildlife Center.

Figures

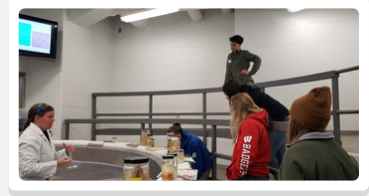
Fig. 1. Semi-firm cutaneous nodule at the base of the left pinna with extensive ulceration and crusting. A similar nodule is present at the base of the right pinna (not pictured).

Fig. 2. A dense population of atypical histiocytes infiltrates and expands the dermis and surrounds the pinnal cartilage. Note areas of epidermal ulceration and crusting (arrowheads). Fig. 3. Dense sheets of atypical histiocytes in the dermis separated by dermal collagen and adnexa. HE.

Fig. 4. Small numbers of multinucleated giant cells interspersed within the histiocytic population (arrowheads). HE. Fig. 5. Diffuse cytoplasmic immunolabeling of Iba-1 in atypical histiocytes. Iba-1-IHC.

Dane County 4-H at the WVDL - Mock Herd Outbreak Investigation

On November 20, 2022, WVDL hosted a group of high school students from the Dane County 4-H chapter to participate in a mock herd disease outbreak investigation. Students learned about necropsy, parasitology, bacteriology, histology, and carcass disposal as well as different roles involved in an infectious disease outbreak situations in veterinary species. Thanks to the WVDL Pathology Sciences team, Pathologists and Veterinary Client Outreach Specialists for putting on a great event! A lot of great learning and fun was had.



Congratulations Lin Schure!

Lin Schure is one of WVDL's amazing Pathology Science Team members and she received an UW - Academic Staff Professional Development Grant. The grant award will allow Lin to travel to South Africa to participate in a 2-week volunteer fieldwork experience with the Shimongwe Wildlife Veterinary Project. There she will have the opportunity to learn more about African animal health issues, conservation efforts, and management of regional infectious diseases. Congratulations Lin! We hope you enjoy this amazing experience. To learn more about African Conservation Experience visit their website: <https://www.conservationafrica.net/>



Serology

Madison Laboratory

Reminder that the WVDL has Discontinued the Brucellosis Complement Fixation (CF) Tests

Upon examination of export health certificates, Brucellosis Complement Fixation (CF) tests are no longer needed. The Brucellosis CF test has been discontinued as of January 1, 2023. For Brucellosis serological assays, the WVDL offers a variety of tests including the Brucellosis Buffered Acidified Plate Antigen (BAPA; also called Brucellosis Buffered Antigen Test (BBAT)), Antibody Card Agglutination (Card), Standard Tube Test (STT) and the Fluorescence Polarization Assay (FPA) tests. Please check the export health certificate requirements for the country or state that the testing is needed for. The name of the tests may vary slightly from country-to-country. Please call the WVDL if you have concerns about if your requirement can be met by the WVDL testing options. The WVDL will automatically perform confirmatory testing on any non-negative Brucellosis tests. Please contact the WVDL with any questions or concerns you may have.

Additionally, the John's Disease CF test has already been discontinued several years ago. For John's Disease serological testing needs, please use the John's Disease/ *Mycobacterium avium* subspecies *paratuberculosis* (MAP) ELISA. We also offer the John's Disease/MAP PCR and liquid culture from feces. The John's Disease Liquid Culture is approximately a 9 week culture and should only be used when required for export health certificates.

EHD cELISA Now Available

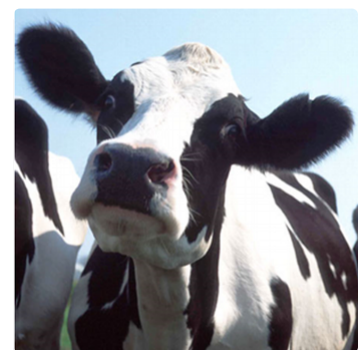
The WVDL-Madison Serology Laboratory has recently validated the Innovative Diagnostics (ID Vet) Epizootic Hemorrhagic Disease (EHD) competitive ELISA for bovine sera. Previously, the only serological assay available was the EHD agar gel immunodiffusion (AGID), which is required for China's health certificates. However, the EHD AGID demonstrates cross-reactivity with BTV antibodies and thereby produces false positives when serum contains anti-BTV antibodies. Therefore, there was a need to validate the EHD cELISA. The ID Vet EHD cELISA kit performs with a high sensitivity and specificity. **Specificity of the EHD cELISA is superior to that of the EHD AGID.** When sera containing anti-BTV antibodies were tested, they did not show positivity in the ELISA as they did when using the AGID. This kit is also approved for caprine, ovine, and cervid sera; however, the serology lab has not validated those sample types. The test can still be performed on sera from these non-bovine species, but will be reported with a disclaimer. The cost of the test is \$12.00 per sample. Whenever possible, please use the EHD cELISA instead of the EHD AGID.

Interpretation for the EHD cELISA are as follows:

S/N%	Interpretation	Explanation and Recommendation
≥40.00%	Negative	Antibodies specific to EHD virus in ruminant samples were not detected.
30.01 to 39.99%	Inconclusive	Cattle with cELISA results in this range are unknown. It is recommended to retest these animals or submit sample to AGID for confirmation.
≤30.00	Positive	Antibodies specific to EHD virus in ruminant serum samples were detected.

BLV ELISA Testing Reminder

The WVDL has validated the BLV cELISA kit produced by Innovative Diagnostics (ID Vet). We have transitioned away from previous kit manufacturer and are exclusively testing for BLV using the ID Vet cELISA kit. This kit was implemented several months ago and is performing as expected. For inconclusive results, the WVDL recommends testing on the BLV AGID, as needed, at an additional cost.



The ID VET Screen BLV Competition ELISA (BLV cELISA) kit allows for detection of anti-gP51 antibodies in sera from bovine species. Samples are reported as **Competition Percentage (S/N%)**. The S/N% for positive animals is ≤50%.

For bovine serum samples:

S/N%	Interpretation	Explanation and Recommendation
≥ 60.00%	Negative	Antibodies specific to Bovine Leukemia virus in bovine serum samples were not detected.
50 to 60 %	Inconclusive	Cattle with cELISA results in this range are unknown. It is recommended to retest these animals or submit sample to AGID for confirmation.
≤ 50.00%	Positive	Antibodies specific to Bovine Leukemia virus in bovine serum samples were detected.

Serology

Barron Laboratory

Avian Reovirus and *Ornithobacterium rhinotracheale* ELISAs to be Discontinued

Due to very low demand for the Reovirus (REO) and *Ornithobacterium rhinotracheale* (ORT) ELISAs, the WVDL will no longer offer these tests at this time. We ask that you please reach out to our Barron Laboratory, at 715-637-3151, if your flocks requiring this test in the future. We would be happy to discuss the possibility of offering these tests again. If there are any other questions, please do not hesitate to call or email (info@wvdl.wisc.edu).

Sample Collection from Poultry Less Than 3 Days Old for *Salmonella* Testing is Offered at the Barron Lab Location



The WVDL-Barron lab accepts deceased whole body poultry that are less than 3 days old for *Salmonella* culture testing. The sample collection fee is \$11.00 per pool, which may include up to 5 birds. Accessions may be submitted utilizing the General Submission Form or the Avian Tissue Processing Submission Form. *Salmonella* culture and any subsequent grouping and serotyping are performed in accordance with NPIP requirements and billed separately. Per NPIP, WVDL-Barron is required to fully serotype all Group D *Salmonella* isolates. To ensure cost savings when possible, clients may indicate on the submission paperwork if serotyping is not desired beyond Group D *Salmonella* isolates.



EIA/Coggins Testing Information

As spring nears, the WVDL is preparing for yet another busy season of Equine Infectious Anemia (EIA; Coggins) testing. To ensure we are able to continue providing exceptional diagnostic services, we have provided updates and important information about testing in this correspondence.

Updates to EIA Testing Services

1. Official certificates can be expected within 72 hours of receipt at the Barron laboratory.
2. Testing services may be expedited with results available within 24 hours of receipt for an added fee. Please see the website for more information.
3. Submissions that do not meet USDA requirements and require clerical attention for processing will be charged an additional processing fee. Testing services may also be delayed.

Helpful Hints for Frustration-Free EIA Testing

1. The only EIA/Coggins testing available is ELISA.
2. Please submit 1mL of serum, refrigerated and shipped on cold packs.
****Please note, hemolysed serum will be rejected****
3. Samples must be submitted with their completed submission form. Please note, the animal ID listed on the paperwork **MUST** match and be clearly identified on the serum sample.
4. Submitting Veterinarians **MUST** have a valid National Accreditation Number.
5. The WVDL-Barron accepts 3 types of submission forms: the Official Federal VS10-11 form, as well as electronic forms via Global Vet Link and APHIS Veterinary Services Process Streaming (VSPS).

<https://www.wvdl.wisc.edu/wp-content/uploads/2022/02/Equine-Infectious-Anemia-EIA-Submissions-Frustration-Free-Equine-Infectious-Anemia-Testing.pdf>

Please feel free to call (715-637-3151) or email (info@wvdl.wisc.edu) us at any time for answers to your questions.

More information can be found at:

<https://www.wvdl.wisc.edu/index.php/equine-infectious-anemia-virus-eia-diagnostic-testing/>

Save the Date - Swine & Poultry Conference

Swine & Poultry Conference
Held at UW-River Falls Campus
Saturday, April 29th, 2023
8 am – 3 pm

This conference is being held and available to:
Backyard producers, veterinarians & certified veterinary technicians
CE credits will be available
Stay tuned for more information to come!



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