

Winter 2021, Vol. 5, No. 1

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A newsletter about diagnostic trends at the laboratory, animal health topics, interesting cases and new test offerings.

www.vdl.ndsu.edu

Feedback is always welcome. Please send comments, questions and suggestions to ndsu.vetlab@ndsu.edu (specify "newsletter" in the subject line. or call the laboratory at (701) 231-8307.

NDSU Veterinary Diagnostic Laboratory

Director's Corner

We have been fortunate for the mild winter thus far and I know I have certainly not missed moving as much snow as in the preceding two winters. Despite the mild temperatures, the laboratory has stayed consistently busy with our normal caseload in addition to other items brought on by the pandemic.

Of note, the laboratory received its **Clinical Laboratory Improvement Amendments (CLIA)** certificate and began testing human samples for SARS-CoV2 in August. The testing has been a very natural extension of the work we do daily because the testing platforms and technologies are exactly the same as our veterinary tests. We have worked closely with the North Dakota Department of Health in this pursuit and have enjoyed making a small contribution to the pandemic fight.

As spring nears and our caseload begins to increase with calving season, we look forward to another year of working with practitioners, producers and animal owners. We welcome any suggestions and feedback on the services we provide and I encourage you to reach out to us by phone, email or on our website.

Best wishes for 2021,

Brett T. Webb, DVM, Ph.D., DACVP NDSU – VDL Director

Dr. Rice's Mystery Photo



The photo is from an adult domestic longhaired barn cat who was euthanized after losing weight and exhibiting incoordination and neurologic signs. Another cat with similar signs recently died. Rabies examination was negative.

Visit the VDL Website (<u>www.vdl.ndsu.edu</u>) to see the answer and read more about the case.

Calendar: Spring Closures

April 2 – Holiday

May 31 - Memorial Day

NDSU VETERINARY DIAGNOSTIC North Dakota State University

New Faculty and Staff



Dr. Liam Broughton-Neiswanger is the NDSU VDL's newest anatomic pathologist. After serving in the military, Broughton attended Washington State University (WSU) for ee in microbiology

his undergraduate degree in microbiology and master's degree in veterinary virology. His master's thesis was on the molecular epidemiology of ovine progressive pneumonia.

He stayed on at WSU to attend veterinary school, as well as complete a dual anatomic pathology residency/Ph.D. program. His Ph.D. studies focused on genetic and metabolic markers of feline kidney disease. He became board certified with the American College of Veterinary Pathologists in 2019.

Broughton comes to the VDL with a passion for imaging analysis and applying technologic advances to veterinary diagnostics. When not working, Broughton's hobbies include taking the kid camping, playing boardgames with friends (online these days) and working on the '66 Mustang.



Tylise Graff, MLS (ASCP)[™], joined the NDSU VDL on Jan. 26 as a molecular microbiologist in the Molecular Diagnostics (PCR) section. She earned a B.S. degree in medical technology

from Minnesota State University-Moorhead and spent more than 14 years as a medical laboratory technologist, including a number of years as a lead technologist, in the Molecular and Chemistry Send Outs departments. Tylise's experience will be invaluable as the NDSU VDL's Molecular Diagnostics section continues to grow and keep up with the latest technology.

Bench Notes

In addition to T4 testing, the Clinical Pathology section can evaluate **SDMA** (symmetric dimethylarginine) and canine and equine progesterone levels. The new point of care immunoassay system (Micro-Cube from MicroVet Diagnostics) also is capable of determining blood levels of canine CRP, fibrinogen, cortisol, bile acids, fructosamine, pancreatic lipase, serum amyloid A and phenobarbital. Assays will be added to our testing catalogue according to client requests. Please contact us to share which assays are of interest to you.

Toxicology section now performs a **whole-blood test for selenium** by inductively coupled plasma mass spectrometry (ICP-MS). Antemortem whole-blood (EDTA or heparin) selenium is a good indicator of longerterm selenium intake or supplementation. Adequate whole-blood selenium concentrations in most species range from 80 to 350 parts per billion. The NDSU VDL can provide interpretation of whole-blood selenium status of most domestic species, but normal values for exotic species may not be available.

Where's the lesion? Freeze artifact can severely hamper evaluation of tissues and can obscure important tumor features (see Figure 1). During cold winter months with subfreezing temperatures, the addition of alcohol (isopropyl, methanol or ethanol) at a mixture of 1 part alcohol to 9 parts formalin will help prevent freeze artifact in tissue submissions.



Mini Case Reports

Abortion and early neonatal loss comprise much of the winter and spring caseload at the NDSL VDL. As described in the Disease Update section, most of the 2020 abortions were caused by bacterial, fungal or viral pathogens. The three cases presented in this issue are examples of some of the infectious abortion workups performed thus far in 2021.

Case 1

Fetal tissues and placenta from an **Angus calf** that was aborted two weeks prior to the due date were examined. The heifer dam was reported to be lethargic and this was the second abortion on site in a group of about 25 heifers.

The field necropsy did not reveal any gross lesions. Microscopically, however, we found variable regions of mixed inflammation in the lung (Figure 2a). Additionally, we found a placentitis characterized by degenerate neutrophils within the chorion (Figure 2b), along with numerous smudged bacterial colonies within the chorion and cotyledon (Figure 2c). Cultures of the lung, liver and abomasal fluid yielded high numbers of *Campylobacter fetus* bacteria.

Campylobacter abortions in cattle can be due to *C. fetus* spp. *venerealis, C. fetus* spp. *fetus* or *C. jejuni.* The latter two bacteria typically occur sporadically and are transmitted hematogenously from the gastrointestinal (GI) tract of the dam. *C. fetus venerealis* is different in that it is a venereally (sexually) transmitted disease. Bulls are subclinical carriers, while heifers may have higher rates of infertility than older cows.

Further PCR testing identified the isolate to be *C. fetus* spp. *fetus*. Interestingly, the *Merck Veterinary Manual* lists *C. fetus venerealis* and *C. fetus fetus* as causes of bovine genital campylobacteriosis. Experimental infection is necessary to determine if a particular strain of *C. fetus fetus* is associated with infertility.

Figure 2: Campylobacter abortion in a bovine fetus. We found mixed inflammation in the lung (a), as well as within the chorion of the placenta (b). Also within the placenta are many smudged bacterial colonies (c). (H. Pecoraro, VDL pathologist).

More cases on page 4



Mini Case Report

Case 2

The next case is from a frozen **Angus fetus** that was aborted several days prior to submission. The dam was an older adult cow. Other than evidence of predation, no gross lesions were noted. No placenta was available for evaluation.

Under the microscope, most of the tissues also were unremarkable, except for the lung and thymus. Within pulmonary alveoli were aggregates of histiocytes and neutrophils (Figure 3), while mild lymphoid depletion was observed in the medullary thymus. All cultures were negative for pathogenic bacteria, and PCR for common causes of abortion – IBR, BVDV and *Leptospira* spp. – also were negative. Given the histiocytic alveolitis, *Ureaplasma diverticulum* was suspected and confirmed by PCR.

Ureaplasmosis causes a unique placentitis. However, placenta was not available and placentitis could not be determined in this case. Confounding this fact, other etiologic agents can be associated with a similar pattern of inflammation in lung tissue. In addition, *U. diverticulum* is a ubiquitous organism as it is a commensal of the respiratory and GI tracts of cattle. Diagnosis in this case was based on the lesion plus positive PCR from pooled sterile lung, liver, thymic and kidney tissues.



Figure 3: Ureaplasmosis in an aborted bovine fetus. We found small aggregates of histiocytes and neutrophils within the alveoli of the lung. (H. Pecoraro, VDL pathologist).

Case 3

The final case is **two stillborn lambs** who likely died during dystocia. The dam was euthanized due to uterine rupture while lambing.

Grossly, multiple limbs of both lambs were malformed with muscle contractures along various joints, medically referred to as arthrogryposis (Figure 4). We also found scoliosis (sideways curvature) and kyphosis (upward bending) of the spine of one of the lambs.

For both, the cerebellum was markedly smaller than normal for a full-term lamb (cerebellar hypoplasia). Edema also was present around the head and neck of one of the lambs, consistent with dystocia. Other than mild hemorrhage in the adrenal gland of one of the lambs, no significant lesions were observed microscopically.

The constellation of cerebellar hypoplasia and arthrogryposis in a lamb is highly suspicious for *in utero* viral infection such as Border (related to BVDV), Cache Valley, La Crosse, Akabane, Schmallenberg and Wesselsbron diseases. Scientists think that *in utero* infection affecting the fetal brain leads to the inability of

> Figure 4: Arthrogryposis in a stillborn lamb. (Photo courtesy of the case submitter).

the fetus to move appendages. This lack of movement causes contractures around joints and, thus, the malformation seen with arthrogryposis.

Cache Valley irus (CVV), a mosquito-borne virus, has been diagnosed in sheep at the NDSU VDL and was suspected in this case. Blood from the dam was positive for serum antibodies to CVV. Thus, evidence indicates these lambs may have been infected early in gestation. Infection would have occurred in the late summer/early fall while mosquitoes still were active in the region.

CVV can affect humans; however, according to the Centers for Disease Control and Prevention, fewer than 10 cases ever have been reported. The virus has been detected in several mosquito species in North and Central America.



Disease Updates

Last winter's newsletter (Vol. 4. No 1) reported significant differences in the unknown cause of **bovine abortions** for field-(59%) versus lab-performed necropsies (37%) during 2019. The main contributor to this difference was likely submission of an incomplete set of tissues for abortion workup. However, while evaluating the abortion data for 2020, another potential factor has emerged – namely, nutritional deficiencies and toxicities.

For the year 2020, 75% of all bovine abortions were assigned to at least one etiology (Figure 5a). Of the 152 cases submitted from January through December, 62 (41%) were lab-performed necropsies, while 90 (59%) were submitted from the field. The percentage of cases with an unknown cause of abortion were similar for lab-performed and field necropsies (26% versus 25%, respectively).

This overall reduction in unknown abortion etiology may be due to the identification of nutritional deficiencies or toxicities in 31% (47 of 152) of aborted calves. Interestingly, 28% (13 of 47) of these were diagnosed with a concurrent pathogen. Copper and vitamin A deficiencies, determined by liver analysis, were the most common abnormalities.

Copper deficiency has been associated with increased risk to pathogens in neonatal and young cattle, while the effects

of vitamin A deficiency are well documented in young calves, including low growth rates, weakness, trouble nursing and blindness.

Colostrum and milk are primary sources of neonatal vitamin A. Low concentrations are expected in the bovine fetus, given the lack of vitamin A intake. Furthermore, the relationship between vitamin A deficiency in the fetus and abortion has yet to be established in bovids.

Thus, vitamin A deficiency in the dam may be a more sensitive marker to determine whether an abortion is possibly vitamin A related and submission of maternal serum with aborted tissues is recommended for vitamin A analysis. Maternal serum also is useful in ruling out exposure to other infectious diseases.

Despite the high numbers of nutritional abnormalities, pathogens (Figure 5b) remain the main cause of abortion in cases submitted in 2020 at 47% (72 of 152). Bacterial and fungal organisms were identified in more than half of infectious abortions (38 of 72; 53%). Interestingly, another case of *in utero* liver fluke infestation was noted. In many cases (21 of 72, 29%), we found evidence of infection (for example, pneumonia, hepatitis or placentitis), but a specific pathogen could not be identified. What is important to remember is that we have **three factors in abortion** to consider: *fetal, maternal and placental.* The more factors that are evaluated, the more likely a diagnosis can be determined.

As we head into abortion season, the following specimens should be submitted for optimal abortion work-up results:

- Fresh and formalin-fixed placenta
- Whole fetus/neonate (recommended) or fresh and fixed tissues, including:
 - Fresh brain, heart, lung, liver, kidney, spleen, abomasal fluid, thymus, thyroid, lymph node, fetal eyeball/vitreous humor (for nitrate analysis) and any tissue with a suspected lesion
 - Formalin-fixed brain, thymus, heart, lung, liver, kidney, spleen, conjunctiva, adrenal gland, skeletal muscle and any tissue with a suspected lesion
- Feed and water
- Maternal sera (acute and convalescent)

Figure 5: 2020 abortions listed by overall cause (a) and infectious agents (b).



NDSU Veterinary Diagnostic Laboratory

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Staff Spotlight

Many of you have met Diandra Gallagher, our receiving, necropsy and clinical pathology technician, while dropping off samples. Besides being a wearer of many hats, Diandra also is a licensed veterinary technician with years of clinical experience before joining the NDSU VDL staff in July 2011. She graciously agreed to be this issue's staff in the spotlight.

What fictional world or place would you like to visit? I am reading a series that takes place in the future right now, so that would be cool to see.

If you could bring back any fashion trend, what would it be? Stirrup pants!

Do you have a favorite plant? I kill them, so no.

What would your superpower be and why? To be invisible so I could listen in on what people were talking about.

Are you a morning person or a night person? Night

Popcorn or M&Ms? If we are talking movie popcorn, then I would go for that. But M&M's have come out with some good new flavors like brownie, so I guess it's a toss-up.



Diandra Gallagher (Photo by Kelly Benson, VDL chemist)



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Contact Information

NDSU Veterinary Diagnostic Laboratory P.O. Box 6050 NDSU Department 7691 Fargo, ND 58108-6050 Phone: (701) 231-8307

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