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In This Issue

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

www.vdl.ndsu.edu

We welcome comments, questions and suggestions. Please email us at vetlab.ndsu@ndsu.edu or call the laboratory at (701) 231-8307.

NDSU Veterinary Diagnostic Laboratory

I was pleased to see many practitioners and technicians at the VDL's annual continuing education event held May 21. The program, headlined by lectures from the University of Wyoming's Dr. Donal O'Toole on chronic wasting disease and bovine herpesvirus-1 abortion, was well-received.

This spring, the North Dakota Legislature appropriated funding for a new veterinary diagnostic laboratory facility. The new facility, to be constructed during the next two years, will provide desperately needed laboratory space and allow for expansion of testing services. We extend our thanks to laboratory users and other stakeholders whose support and work on our behalf over the past few years was integral in making the new facility a reality.

After a very quiet 2014, the state had its first case of anthrax in more than two years. Also, a case of tularemia was diagnosed in a squirrel this summer.

Lastly, don't forget the annual North Dakota Veterinary Medical Association meeting, to be held in Medora, is right around the corner on Aug. 9-11. For those planning on attending, please visit the VDL's booth and complete a short survey for a chance to win an iPad. Dr. Claire Miller, the VDL's microbiologist, will be speaking on important topics in large animal infectious disease. Have a wonderful summer!

Sincerely,

Brett T. Webb, DVM, PhD, DACVP
Veterinary Pathologist
NDSU Veterinary Diagnostic Laboratory

NDSU VETERINARY DIAGNOSTIC LABORATORY
North Dakota State University

Anthrax

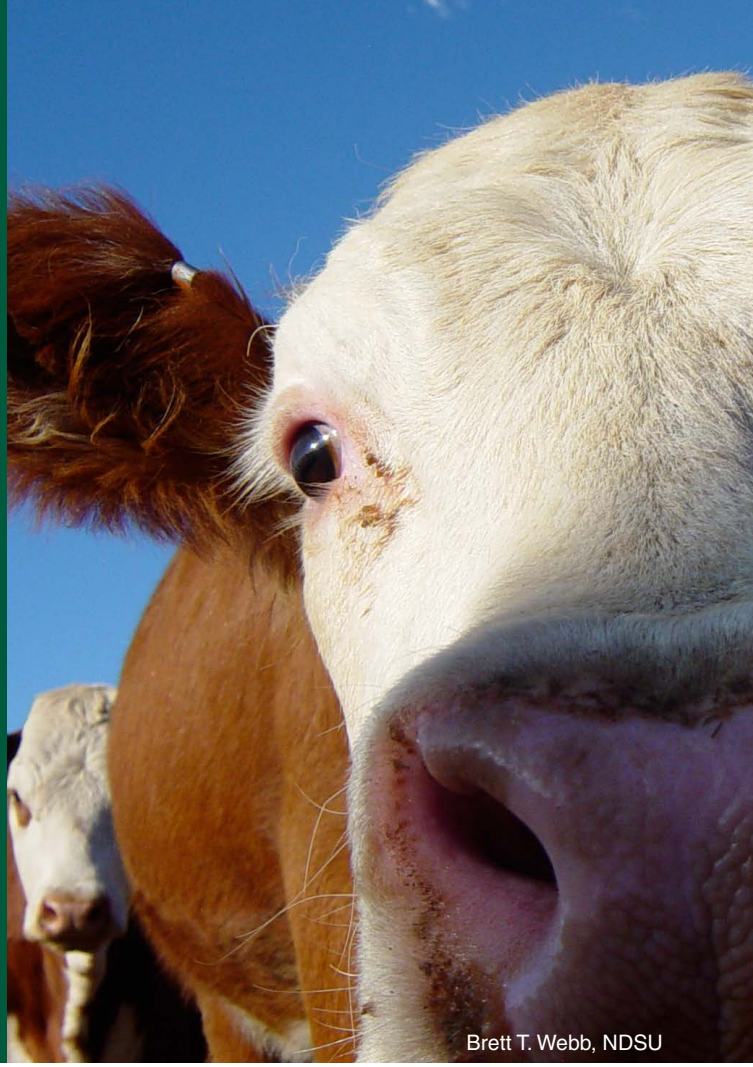
A cow in northwestern Grand Forks County tested positive for *Bacillus anthracis* in mid-June. This was the first case of anthrax diagnosed by the VDL since the summer of 2013. It serves as a reminder to consider this disease in cases of cattle found dead without displaying clinical signs.

The laboratory primarily uses a PCR assay to test for anthrax, although occasionally, a combined approach of culture and PCR is needed to confirm the presence of this bacterium in poor-quality specimens.

The preferred sample is a minimum of 0.5 ml of whole blood obtained from a head, neck or tail vein. If blood is unavailable, other tissue samples such as an ear notch or, if the carcass is open, body cavity fluids or spleen tissue can be used.

When collecting samples from animals suspected of having died of anthrax, direct particular attention toward dressing any cuts or abrasions in addition to the use of double gloves, apron/coverall and disposable boot covers because greater than 95 percent of human anthrax cases involve the cutaneous form.

The VDL offers anthrax sampling kits free of charge. To request a kit, please call the laboratory at (701) 231-8307 or email us at ndsu.vetlab@ndsu.edu.


Brett T. Webb, NDSU

Diagnostic Laboratory Calendar

Aug. 9-11 – Annual North Dakota Veterinary Medical Association Meeting

Monday, Sept. 7 – Labor Day – Laboratory closed

Sept. 24-26 – North Dakota Stockmen's Association Meeting

Wednesday, Nov. 11 – Veterans Day – Laboratory closed

Thursday, Nov. 26 – Thanksgiving Day – Laboratory closed

Thursday, Dec. 24 – Christmas Eve – Laboratory closes at noon

Friday, Dec. 25 – Christmas Day – Laboratory closed


Brett T. Webb, NDSU

New Tests

The VDL again is offering serum neutralizing assays for BVDV type 1, BVDV type 2, IBRV, BRSV and PI_3. The cost for each assay is $7, and the assays are run weekly.

A bovine abortion serology screen also is available and includes BVDV type 1, BVDV type 2, IBRV, six *Leptospira serovars* and *Neospora* for $40.

Bovine respiratory multiplex – BVDV, BHV-1, BRSV, BCoV (respiratory) $45.

Visit our website (www.vdl.ndsu.edu/tests) for more information.

Submission Tips

Need Quicker Results But Can't Wait for a Report? Get Online Access Now

Online access to your account allows you to view test results as soon as they are entered into our laboratory information system without having to wait for a report. To request a login, please visit www.vdl.ndsu.edu and click the login tab, then select "sign up for an account."

A Little Ink Goes a Long Way

Accurate information concerning surgical margins and completeness of excision is critical to appropriate management of malignancies. Inaccurate surgical margin assessment can lead to unnecessary surgery to obtain larger margins and thus additional cost and morbidity.

Inking is an easy and quick technique that allows the surgeon to indelibly mark the true surgical margins to ensure that margin size and completeness of excision are reported accurately.

True surgical margins can be difficult to determine accurately in histologic sections because specimen contraction or curling during fixation, shape and orientation, and whether the tissue block is fully faced in, can have significant effects.

Pathologists look for features such as cautery, hemorrhage and other artifacts to determine whether the margin they are viewing in the histologic section represents a true surgical margin. These features often are variably present and sometimes simply absent or unclear.

In these latter cases, completeness of excision and margin size typically represent an educated guess or, if completely uncertain, a statement that margins can't be determined accurately. Inking surgical margins removes this ambiguity.

India ink or other permanent inks work quite well and are widely used, but special tissue-marking ink can be easier to use and gives better results. The VDL offers free tissue ink to practitioners interesting in inking their masses. Contact the laboratory if you are interested.

For detailed instruction on inking masses, visit www.vdl.ndsu.edu/images/uploads/page_files/Use_of_tissue_dye_to_denote_surgical_margins1.pdf.

Video Conferencing with Pathologist Offered

The NDSU-VDL is offering free consultation with pathologists via FaceTime or Skype for practitioners faced with challenging cases. The service is being offered on a trial basis, and its continuation will be subject to a sufficient level of practitioner interest.

If you find yourself necropsying an animal with unusual lesions, are unsure of what process is at hand or need sampling recommendations to cover all possible differentials, we are here to help.

To videoconference from your smartphone or other compatible device, please call the laboratory at (701) 231-8307 so a pathologist can be located and a connection established. In most instances, a pathologist can be made available immediately, but practitioners are encouraged to call ahead of time to ensure availability. The service is limited to use by veterinarians.

Reminder: Tritrichomonas Testing

The VDL offers pooled *Tritrichomonas fetus* PCR on preputial washes. The samples will be pooled in groups of five at the laboratory. Just be sure to request pooled testing on the submission form.

The submitter is responsible for checking destination state requirements regarding the acceptability of pooled test results.

Tritrichomonas PCR testing days have changed. **The assay now is run on Tuesday and Thursday.** Samples must be received by 11 a.m. of the test day or they will be held until the following scheduled test day.

Sample Collection Recommendations for Bacteriology

Claire Miller DVM, PhD, DACVM

Obtaining high quality **diagnostic** samples is critically important for determining treatment and prognosis in clinical cases. Low quality samples can be a waste of money at best or provide misleading and erroneous information at worst.

Here are some guidelines to aid in sampling, choosing transport media and shipping conditions. Please contact the laboratory with specific questions about sampling at (701) 231-8307.

General Sampling:

- Adequate sample size increases our ability to recover potential pathogens.
- Fresh tissue, fluids and feces are preferred over swabs. Ideally, at least 1 ml of fluid, 3 cm^3 of tissue or 5 g feces should be submitted.
- Swabs are best used for eye, uterine and deep wound infections. Submitting multiple swabs of the same area increases our bacterial recovery ability.
- Attempt to sample at the interface of normal/abnormal, if applicable.
- **Do not submit dry swabs** unless specifically designed for maintenance of bacterial viability.

Transport Systems:

- Tissue:
  - Sterile tube or whirl-pak bag – Small tissues that are at risk of drying out can be submitted in a small amount of nonbacteriostatic fluid (sterile saline) in a sterile container. Port-a-cul® systems also work well, with tissue placed into the semi-solid agar.
- Fluids:
  - Sterile tube or Port-a-cul® vial – The tube should not contain anticoagulant or other product.
- Feces:
  - Leak-proof container (urine collection cup, others) – This allows for ease of sample processing.
- Swabs (aerobic culture):
  - Commercial bacterial transport system (see below) – Alternatively, a swab may be placed in a (0.5 to 1 ml) nonbacteriostatic fluid (sterile saline) in a sterile red top tube.
- Anaerobic:
  - At least 1 ml of fluid or ≥3 cm^3 of tissue, which can be transported as described above – If small samples or swabs need to be cultured anaerobically, the samples should be placed into an anaerobic transport system (Port-a-cul®, others).

Shipping Conditions:

- Most samples should be shipped chilled but not frozen.
- Blood cultures, cerebral spinal fluid samples, anaerobic-only samples and fungal cultures should be maintained at room temperature.
- Overnight shipping is recommended.

Suggested Products:

- Aerobic culture:
  - BD® BBL® CultureSwab® EZ and EZ II
  - BD® BBL® CultureSwab® Plus Aimes Medium (can be with or without charcoal)
  - BD® BBL® CultureSwab® Plus Liquid Stuart Medium or Liquid Aimes Medium
- Anaerobic culture:
  - BD® BBL® Port-A-Cul® tubes, vials or jars
- Mycoplasma:
  - BD® BBL® CultureSwab® Plus Aimes Medium without charcoal
  - BD® Universal Viral Transport System

Reminder

For herd health submission, please remember to fill out animal IDs on the electronic herd health or serology form. Attached napkins, Post-it notes or other papers are not acceptable. On submissions without completed paperwork, testing will be delayed until the completed forms are received or a $40 surcharge will apply if VDL staff completes the paperwork.

Noteworthy Cases

Listeriosis

Neil Dyer DVM, MS, DACVP

Samples from cattle with signs of central nervous system (CNS) disease are relatively common submissions at the NDSU Veterinary Diagnostic Laboratory (VDL). Such cases are always processed to rule out rabies first for obvious reasons. If a negative rabies diagnosis is received, further testing can be done to look for other sporadic causes of CNS disease in cattle, such as lead poisoning, nervous coccidiosis, brain abscess, polioencephalomalacia (polio) and *Histophilus somni* or *Listeria monocytogenes* infections.

In what has turned out to be a somewhat unusual winter and spring of 2015, the VDL has diagnosed nine cases of listeriosis in cattle since February.

Three of the nine cases presented as reproductive disease (abortion or stillbirth) and the remaining six were all central nervous system cases with evidence of encephalitis. The reproductive cases occurred in well vaccinated herds. There was evidence of fetal pneumonia and placentitis in all cases and recovery of *L. monocytogenes* was managed from lung, liver, abomasal contents and placenta. In two of the three cases, there was a history of feeding silage.

Of the six encephalitis cases, two were bulls and four were cows. All exhibited CNS signs that included circling, blindness, abnormal behavior, belligerence, incoordination, facial paralysis, head pressing, recumbency and paddling. Lesions typical of *L. monocytogenes* encephalitis were observed in brainstem (five) and thalamus (one). *Listeria* was cultured from all but one case, in which the diagnosis was made by histopathology alone (typical lesions).

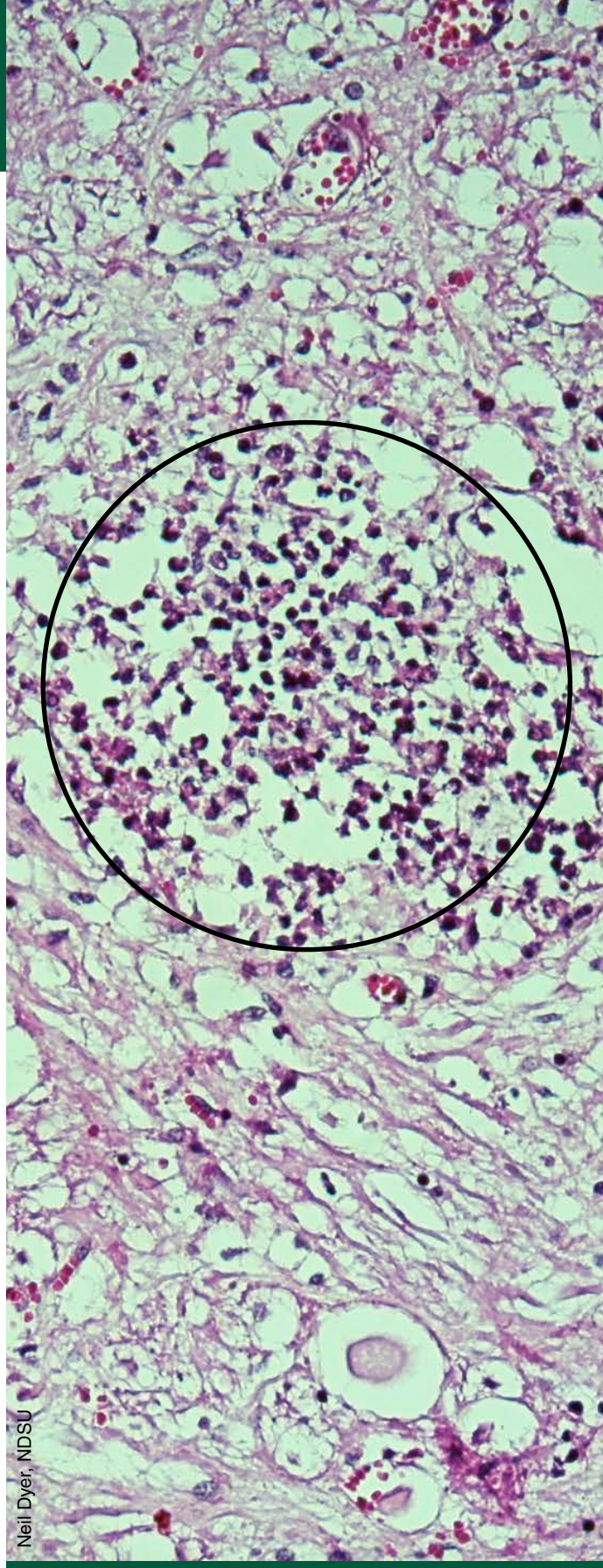
Two cases included respiratory lesions from which bacteria were recovered, indicating a septicemia. The history for these cases was less complete, but a few reported feeding of silage.

The encephalitis cases occurred in March (one), April (two) and May (three). A look at the geographic distribution did not reveal a pattern, but all cases were in central and eastern North Dakota (only one case was west of the Missouri River).

Listeria monocytogenes is a ubiquitous organism that causes infections in a wide variety of birds and mammals (humans included) in more temperate climates. The organism commonly is found in the intestinal tract of carrier animals (particularly ruminants) and the environment, therefore transmission is typically fecal/oral.

Listeria is a gram positive organism that is able to grow in a wide range of temperatures, including 4° C (cold enrichment). The disease is seen more commonly in the winter and spring and traditionally has been associated with feeding of spoiled silage (the bacteria grows well in the less acidic pH).

Listeriosis continued on page 6.



Microscopic section of brainstem, 200X, a Listeric microabscess is within the black circle.

Noteworthy Cases

Listeriosis continued from page 5.

The presentation of disease across species is broad: reproductive disease in all animals, encephalitis in ruminants, septicemia in neonatal ruminants and monogastrics, and hepatitis/myocarditis in poultry. Listeric encephalitis localizes in the brainstem of ruminants. Recovery from disease depends on early intervention, and penicillin is the drug of choice.

Experts disagree on whether animals serve as a reservoir of disease for humans. Despite this uncertainty, it is a good idea to handle all potentially infective material such as fetuses and fetal tissues, fetal fluids, and ruminant brains and milk very carefully. Pregnant women, and older and immunosuppressed individuals should be particularly mindful of avoiding exposure to the organism.

In a typical VDL case workup for CNS disease in cattle, smears from the brainstem and vermis are routed to virology, where the direct FA test for rabies is done. Tissues also are placed in formalin for histopathology.

Following a negative rabies diagnosis, the brain is viewed under the microscope and additional testing is done based on what lesions are observed. Lead cases are sent to toxicology, polio diagnosis is done primarily by histopathology, and *Histophilus* and *Listeria* cultures are set up if inflammation in the brain is observed. Fecal floats can help with potential cases of nervous coccidiosis, and abscesses often are grossly visible.

The reason that North Dakota is experiencing more cases of listeric encephalitis in ruminants this spring is unknown. Case histories provide information that supports the traditional concept that cattle are exposed to high numbers of bacteria in silage. The bacteria then cross the oral mucosa (abrasions) and have access to the central nervous system.

Whatever the reason for the increased incidence this spring, please be reminded that listeriosis is a zoonotic disease as well as rabies and can cause serious disease in humans and animals.

Infectious Canine Hepatitis

A 3-month-old female crossbred dog was presented to the NDSU-VDL for necropsy. The animal was euthanized following a one week history of gastrointestinal signs and sarcoptic mange.

The dog had large areas of alopecia and crusting (mange) on the limbs, muzzle, abdomen and thorax. Grossly, there was mild pulmonary edema. The liver had an accentuated reticular pattern. No gall bladder edema was observed. The serosal surfaces of the intestines were moderately irregular with a ground glass appearance. The remaining organs and structures including the brain were grossly normal.

Histologic lesions were observed in the liver, brain and spleen. Lesions within the liver consisted of random and periacinar foci of hepatocellular necrosis with high numbers of large, amphophilic intranuclear inclusion bodies within hepatocytes (Photomicrograph 1). The spleen also contained random foci of necrosis and similar inclusions within reticulum cells. Mild meningoencephalitis, primarily affecting the brainstem and cerebellum, was present. Vessels within the neuropil had rare endothelial inclusions. Mild gastroenteritis with scattered necrosis of crypt epithelium and lymphoid depletion also was observed. Eosinophilic intracytoplasmic and intranuclear inclusions were observed in gastric epithelium (Photomicrograph 2).

The gross and histologic changes were consistent with coinfection by canine adenovirus-1 and canine distemper virus. The presence of both agents was confirmed by polymerase chain reaction assays.

Infectious canine hepatitis is caused by canine adenovirus-1. Due to widespread usage of effective vaccines and the fact that the majority of infections are thought to be subclinical or result in mild tonsillitis, cases of infectious canine hepatitis are rarely observed.

In severe cases, clinical signs include vomiting, melena, high fever and abdominal pain. Jaundice, pale mucus membranes and neurologic signs also can be observed. Clinicopathologic abnormalities can include severe leukopenia, thrombocytopenia, hyperbilirubinemia, elevated ALP, elevated ALT, proteinuria and prolonged APTT. Blue eye or corneal edema, referable to a type three hypersensitivity, is seen only in the convalescent stage, typically two to three weeks following infection.

As the virus has tropism for endothelial cells and hepatocytes, gross lesions of hemorrhage on the serosal surface of abdominal organs, an enlarged and mottled liver with small fibrin strands on the surface and edema of the gall bladder wall are common.

The pathogenesis of infectious canine hepatitis is incompletely understood. Initial entry is thought to occur orally and possibly through inhalation following exposure to primarily urine of infected dogs. Initial viral replication occurs in tonsillar macrophages and dendritic cells. Leukocyte trafficking and possibly a cell-free viremia of short duration lead to dissemination of the virus.

Hepatic necrosis begins approximately one week after infection. Progressive viral-induced cytolysis of hepatocytes and endothelial

cells causes consumption of coagulation factors, which is responsible for the presence of widespread hemorrhages on serosal surfaces.

Neurologic disease in infectious canine hepatitis is thought to be mediated by endothelial cell damage within the brain and subsequent hemorrhage rather than direct viral effects on neurons and glial cells. Although the virus is largely cleared from the liver around day 10 post infection, it is shed in the urine for many months.

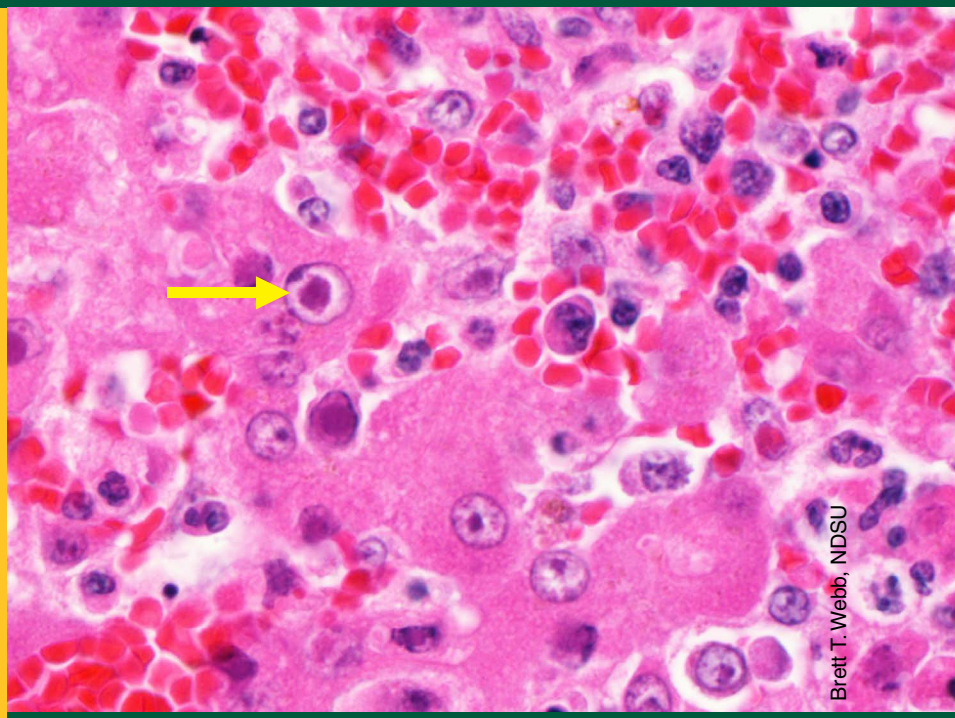
Clinical cases of canine distemper virus are not uncommon, and encephalitis with or without pneumonia and enteritis typically is observed. The presence of coinfection with canine distemper and adenovirus in this animal may suggest immunosuppression or a predisposition to infection or development of disease following infection by the other virus type. What is unclear in the present case is which virus the dog may have contracted first.

Antemortem diagnosis can be achieved by submitting oropharyngeal and ocular swabs for RT-PCR/PCR for canine distemper virus and adenovirus, respectively. The molecular diagnostics section of NDSU-VDL offers assays for many canine infectious diseases, including canine adenovirus 1, adenovirus 2, distemper virus, parvovirus and corona virus.

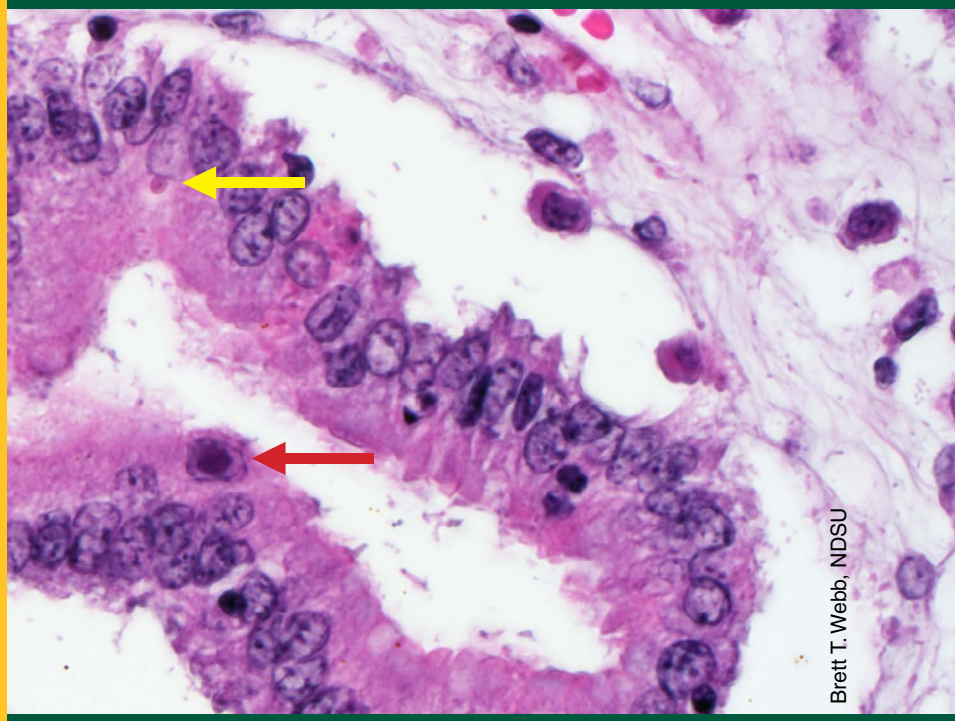
When submitting samples for antemortem diagnosis of infectious disease, understanding that the optimal sample can vary depending on the disease agent and stage of clinical infection is important.

Due to the relative short duration of viremia in many of these diseases, testing of only whole blood can lead to false negative results. Combined testing of oropharyngeal swabs and whole blood or feces (for enteric viruses) is recommended. In most cases, swabs and whole blood can be pooled to reduce testing costs.

Please visit www.vdl.ndsu.edu/tests or contact the laboratory at (701) 231-8307 for more information.



Photomicrograph 1. Large numbers of intranuclear inclusion bodies in hepatocytes due to canine adenovirus type I infection.



Photomicrograph 2. Gastric epithelium with intracytoplasmic inclusion bodies (distemper virus)(yellow arrow) and intranuclear inclusion body (red arrow) (adenovirus).

Noteworthy Cases

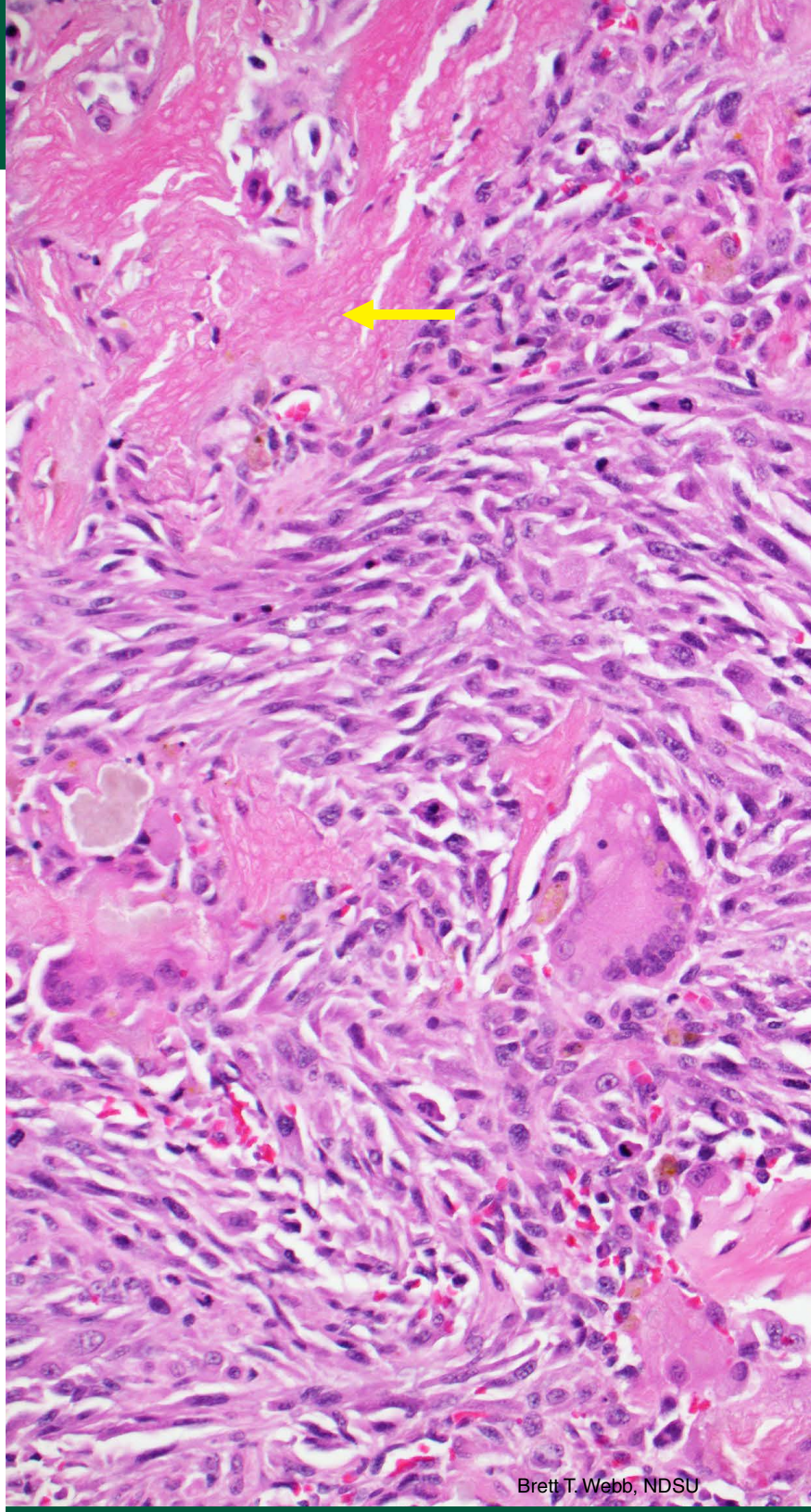
Extraskeletal Osteosarcoma Arising From a Pilomatricoma

A subcutaneous mass on the lateral abdomen of a 13-year-old female Cairn terrier was submitted to the VDL for histopathology. The mass had been present for many years but recently increased in size and was suspected to have become infected.

Histologically, the mass was well demarcated and consisted of interweaving sheets of spindle cells forming irregular osteoid that surrounded large masses of partially mineralized keratin with ghost cells. Small areas of necrosis infiltrated by neutrophils, as well as peripheral aggregates of lymphocytes, also were present. The mass appeared completely excised. The histomorphologic features were consistent with an extraskeletal osteosarcoma arising from a pilomatricoma.

The mammary gland is the most common site for extraskeletal osteosarcoma, which also have been described in the gastrointestinal tract, spleen, skin, muscle, eye, liver, thyroid and urinary tract. In the esophagus, development of extraskeletal osteosarcoma occurs secondary to *Spirocera lupi* infestation.

Extraskeletal osteosarcoma tends to affect older dogs and, overall, the prognosis is poor with median survival times less than osteosarcoma arising within the skeleton. Malignant transformation of the mesenchymal component of epithelial tumors is well-documented but is not commonly observed, with the exception of mammary gland tumors.



Photomicrograph of tumor showing keratin ghost cells (arrow) and malignant osteoblast.

Cover photo by Brett T. Webb, NDSU

Contact Information

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For more information on this and other topics, see **www.vdl.ndsu.edu**