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Diagnostic Laboratory
Calendar

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

The NDSU-VDL newsletter is back

thanks to VDL's new pathologist, Dr. Heidi Pecoraro, who graciously agreed to help resurrect the publication.

The past year has brought significant changes for the VDL with adjusting to the new facility, the retirement of longtime pathologist and former director Dr. Neil Dyer, and hiring Dr. Pecoraro. The laboratory is pleased to have been able to recruit someone of Dr. Pecoraro's caliber, and I am confident you will enjoy working with her. She has a diverse background and brings a variety of expertise to the position.

For those who were not able to make the laboratory tour at the August North Dakota Veterinary Medical Association meeting, please feel free to stop by for a tour of the new laboratory anytime.

I hope you enjoy the articles and news items enclosed in the newsletter. From our laboratory to your practice, we wish you a wonderful and safe new year.

Sincerely,

Brett T. Webb, DVM, PhD, DACVP
VDL Director and Veterinary Pathologist

NDSU VETERINARY DIAGNOSTIC
LABORATORY
North Dakota State University

Testing Highlights

Ergot Alkaloid Analysis by LC/MS/MS

Michelle Mostrom, DVM, MS, PhD, DABVT, DABT, VDL Veterinary Toxicologist

The North Dakota VDL is offering ergot alkaloid analysis of cereal grains (wheat, barley, rye) and grasses/hays by LC/MS/MS (liquid chromatography coupled with tandem mass spectrometry) for the main *Claviceps* ergot alkaloids: the ergopeptines (-ines, biologically active), including ergotamine, ergosine, ergocristine, ergocryptine and ergocornine, and their isomers, the ergopeptinines (-inines, biologically inactive). In nature, ergopeptinines always accompany ergopeptines, and during storage of raw materials through time or improper storage, more ergopeptinines may form.

The conversion of -ines to -inines can occur under certain conditions, and -inines can be reactivated to -ines. Therefore, both epimers should be determined for ergot alkaloid contamination in animal feeds.

The current Official U.S. Standards for Grains regulatory guidelines for ergot contamination in grains is a crude measurement and determines the percentage of ergot sclerotia in a sample. However, a consistent relationship

between the ergot alkaloid concentration in the sclerotia and total ergot alkaloid concentration in grain samples cannot be established.

Generally, concentrations of ergot alkaloids between 200 to 800 parts per billion (micrograms per kilogram) in the total ration can be considered a threshold level to cause ergotism in livestock species. A representative sample of the cereal grains or hay with seed heads of about 1/2 to 1 pound is needed to dry and grind for analysis (minimum of 50 to 100 grams). Please see the VDL website (www.vdl.ndsu.edu) for test information.

The increased use of no-till farming and leaving vegetation and sclerotia on the soil surface for successive crops, consumer desire to utilize organic foods without chemicals, and dramatic spring and summer wet-weather events will tend to increase the incidence of *Claviceps purpurea* in cereal crops and grasses and exposure to ergot alkaloids.



Samples of ergot contamination of triticale (left) and wheat (right). (Brett Webb, NDSU)

Bone Marrow Fat Percentage in Large Animals by Air-dry Technique

Michelle Mostrom, DVM, MS, PhD, DABVT, DABT, VDL Veterinary Toxicologist

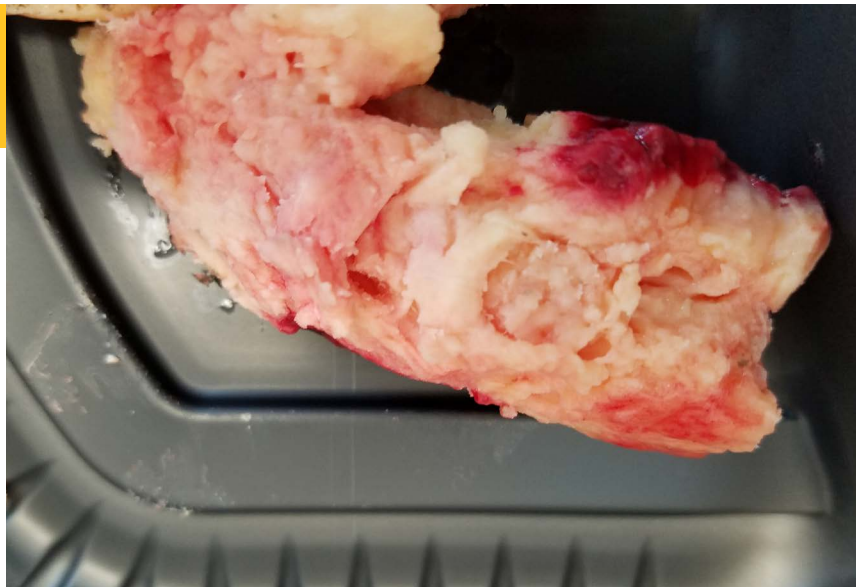
In cases of severe livestock emaciation or starvation, bone marrow fat may support a diagnosis of depletion of whole-body energy reserves. Bone marrow fat estimates the residual fat in bone marrow using an air-dry technique.

Typically, normal large animals have more than 80 percent fat in the bone marrow, and animals suspected of death by starvation have less than 20 percent residual fat. In northern states, if animals die in the winter and freeze, intact femur bone can provide adequate bone marrow for analysis for months after death if carcasses are frozen and no damage to intact bone occurs from predation.

The femur is the bone of choice to evaluate residual fat. The humerus also can be used if adequate bone marrow is available. If veterinarians have difficulty removing bone marrow from the femur, the entire femur can be submitted to the lab and the lab will remove the bone marrow. Please call the lab in this case.

Submit about 30 grams of bone marrow from the medullary cavity (no medullary bone). Young animals may have a smaller sample volume. If necessary, sample both femurs to acquire enough bone marrow.

Place sample in a zip-top bag, seal well and keep refrigerated before shipping the sample with an icepack. Prevent damage during transit by placing the sample in a solid container.



Normal bovine bone marrow is whitish, firm and greasy, with about 80 percent bone marrow fat (top), compared with the gelatinous bone marrow, with less than 10 percent bone marrow fat from a malnourished bovid (bottom). (Michelle Mostrom, NDSU)

Testing Highlights

Lower Pricing on Bovine Leukemia Virus (BLV) PCR testing

The VDL now offers lower pricing on bovine leukosis virus (BLV) PCR testing.

Serology remains the best and most cost-effective test to screen animals for BLV infection, but in herds working toward reducing or eliminating BLV infections, the laboratory recommends PCR testing of seronegative animals to identify those with low levels of BLV, who may have not yet seroconverted.

Cost of first PCR test.....\$25
Each addition PCR test\$15

Please see the BLV test page (www.vdl.ndsu.edu/tests/bovine-leukosis-virus-blv-pcr) for sample requirements and test schedule.

Low-cost Next-day Shipping with UPS

We have partnered with UPS to provide an affordable and reliable way to get your samples to the VDL in one day. You can purchase prepaid, pre-addressed UPS Ground and Next Day Air Labels.

\$7 Flat Rate Ground Shipping

\$15 Flat Rate Next Day Air Shipping

For more information or to order labels, call 701 231-8307

Submission Tips

Submission Form

Be sure to fill out all pertinent information when submitting samples to the VDL. Especially, animal information, client information, clinic information, tests desired and clinical history, if known, should be included. When applicable, samples from multiple animals submitted together should be marked clearly on the submission form and samples.

Tissue Fixation in Winter Months

The addition of alcohol (isopropyl, methanol or ethanol) at a mixture of one part alcohol to nine parts formalin will help prevent freeze artifact in tissue submissions.

Johne's Sample Submission Tips

To ensure the greatest accuracy and speedy turn-around time for your clients, please review our Johne's sample submission tips.

1. Enter animal IDs on the electronic herd submission form. Visit the laboratory's website (www.vdl.ndsu.edu/forms) to download the form. The form can be submitted electronically to the VDL by emailing ndsu.vetlab@ndsu.edu.
2. Send at least 5 grams (about 1 teaspoon) of feces in a double-knotted sleeve labeled with permanent marker (sample number **and** animal ID). Other acceptable containers are screwcap plastic jars or zip-top bags. No blood tubes, Whirl-paks or exam gloves.

For additional information, see our complete Johne's guide (www.vdl.ndsu.edu/resources).

Noteworthy Cases

Goiter in Beef Cattle

Teresa Newell, DVM, PhD, DACVP, VDL Veterinary Pathology

Trace elements, although required in minute quantities, are essential for maintenance of health, productivity, growth, reproduction and immunity. Iodine, a trace element found in soil, is an essential component of thyroid hormones vital to the regulation of metabolic processes. Iodine deficiencies in beef cattle are rare but may be associated with reduced fertility, goiter, stillbirths and birth of weak, sometimes hairless calves.

The VDL typically sees occasional cases of congenital goiter in small ruminants, but diagnosis of iodine-deficient goiter beef cattle has been exceedingly rare. However, iodine deficiency was identified in two regional beef herds experiencing stillbirths and weak calves during the spring of 2018.

The index case, a full-term, normally developed but weak Simmental-Red Angus calf, was presented after death at 1 day of age. No diagnostic macroscopic lesions were detected (thyroid gland minimally/equivocally enlarged). Histologic examination revealed generalized marked follicular epithelial hypertrophy with infolding of epithelium into follicular lumens and scant pale-staining colloid.

Fresh thyroid tissue was sampled from a fetus submitted from the same herd approximately 10 days later, at which time five of 17 calves from the herd reportedly had been stillborn or weak and nonviable. ICP liver mineral analysis on the fetus was within normal limits; however, a profoundly deficient level of 86.7 micrograms per gram ($\mu\text{g/g}$) of iodine was identified on a thyroid iodine tissue assay (reference range 1,100 to 1,800 $\mu\text{g/g}$). Additional history indicated no salt or mineral supplementation was available to the herd.

In a subsequent case, two stillborn calves were submitted from a beef herd. Bilateral softball-sized goiters, marked anasarca, a cobblestone pattern of hepatic fibrosis, and pulmonary hypoplasia and fibrosis were observed macroscopically in both calves. Severely deficient iodine levels of 8 and 3.5 $\mu\text{g/g}$ were detected in the thyroid gland of the calves, while liver ICP mineral analysis revealed slightly elevated but nontoxic zinc levels (125 and 130 parts per million [ppm]; reference range 20 to 100 ppm).

Additional history indicated 17 of the first 100 calves in the herd were goitrous stillbirths, while an additional 30 of 100 calves were goitrous but viable. Feeding history in the herd again indicated an absence of iodized salt or mineral in the ration. Cows had been fed waste french fries for a prolonged period prior to calving, but subsequent investigations uncovered no information linking fat levels, french fries or other dietary components to long-term iodine deficiency.

Major pathogenetic mechanisms inducing thyroid hyperplasia include iodine-deficient diets, goitrogenic compounds that interfere with thyroid hormone synthesis, genetic enzymatic deficiencies in the biosynthesis of thyroid hormones and, paradoxically, dietary iodine excess. Hence, tissue analysis is indicated in all cases of thyroid hyperplasia and goiter to establish the underlying mechanism.

Bovine fetal iodine deficiency has not been identified previously as a significant factor in abortion/weak neonate work-ups at the VDL, but the 2018 index case in which the thyroid gland was not macroscopically

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abnormal indicates a wider tissue selection, including fixed and fresh thyroid gland and ancillary testing, may be critical to establishing a diagnosis in some cases.

A brief reference regarding iodine supplementation in beef herds can be found at www.agriculture.com/livestock/cattle/health/whens-whys-of-supplementing-iodine-to_280-ar48275.

Noteworthy Cases

Root Crops (Brassica) Associated With Polioencephalomalacia in Calves

Michelle Mostrom, DVM, MS, PhD, DABVT, DABT,
VDL Veterinary Toxicologist

This fall, an acute onset of blindness and lack of spatial awareness/perception occurred in several steer calves. The history was that 360- to 400-kilogram (800- to 900-pound) calves were placed on rotational grazing pastures of beets, turnips, oats and rye under irrigation. The irrigation pivots ran every three days and calves were rotated through pastures every five to seven days.

During the initial three weeks on rotational grazing, calves were gaunt and losing weight, and had diarrheas. After three weeks, calves began to improve in body condition and gain weight, but several calves were noted to appear to be blind and unaware of their surroundings.

The calves were pulled from the pasture and examined. Temperatures were normal, whole blood lead was negative (less than 33 micrograms per liter [$\mu\text{g/L}$]), serum magnesium was high normal at 2.37 milligrams per deciliter (mg/dL), and the cattle water source was low in sulfates at 199 milligrams per liter (mg/L).

Calves were placed back on the rotational grazing pasture, and more calves appeared to be blind. Two steers (one affected chronically and one affected acutely) were euthanized and necropsied. The calves had marked swelling of the cerebrum, with widespread cerebral necrosis and fluorescence of the lesions under ultraviolet light, consistent with polioencephalomalacia.

The rutabaga or turnip (Brassica) forage and tubers contained high concentrations of sulfur, ranging from 0.59 to 0.79 percent (dry-matter basis). The National Research Council recommend a maximum tolerable dietary sulfur level of 0.50 percent sulfur for ruminants on a high-forage diet.



Diagnostic Laboratory Winter Calendar

Monday, Jan. 21 – Martin Luther King Day – Laboratory closed

Wednesday, Jan. 23 – North Dakota Veterinary Medical Association Winter Conference, Bismarck

Tuesday, Jan. 29 – North Dakota Veterinary Medical Association Winter Conference, West Fargo

Monday, Feb. 18 – President's Day – Laboratory closed

Feline Hepatic Lipidosis With Concurrent Renal Disease and Pancreatic Islet Amyloidosis

Heidi Pecoraro, DVM, PhD, DAVCP, VDL Veterinary Pathologist

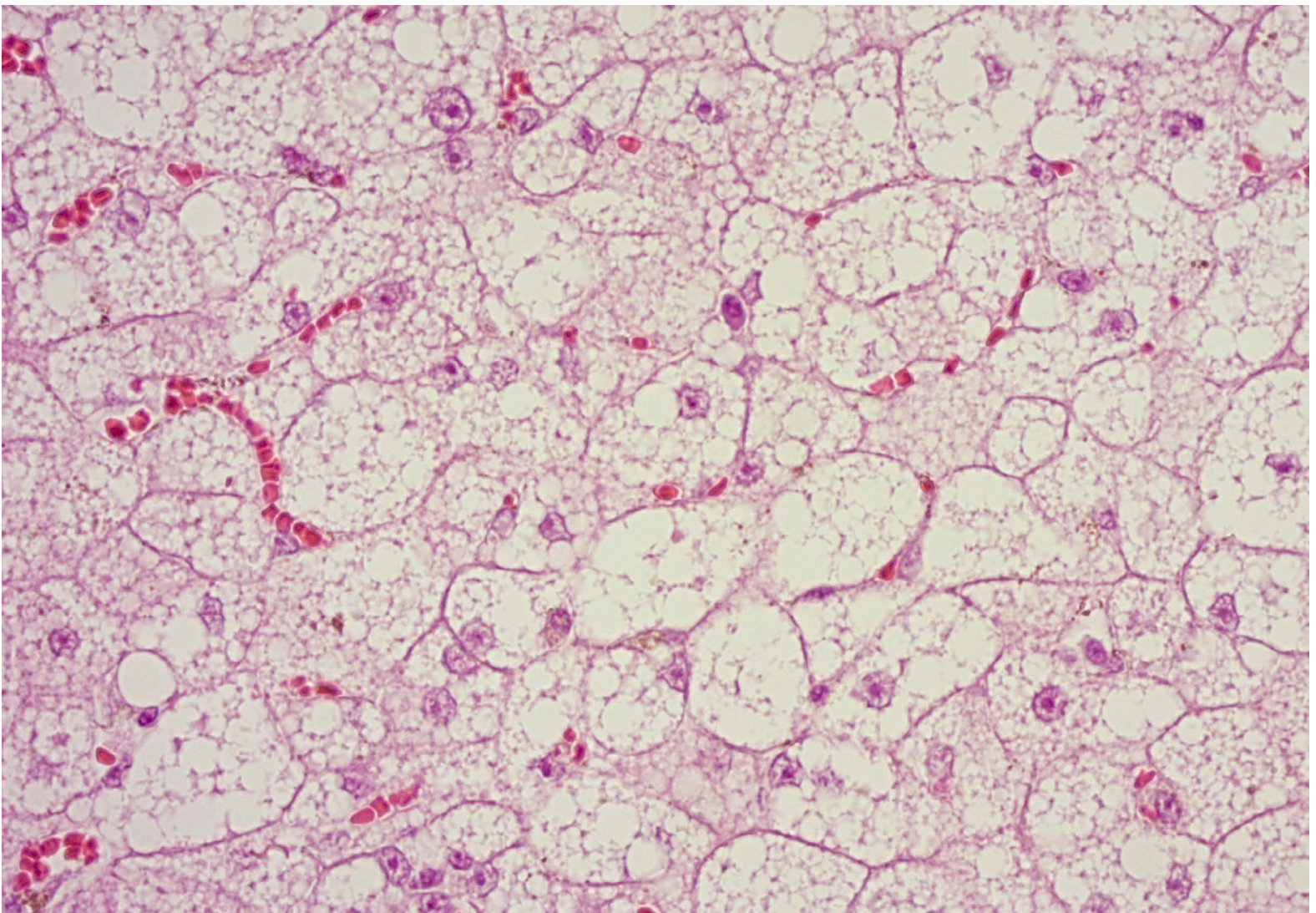
During the holidays, stresses such as boarding while the family is away on vacation or having guests in the home may instigate a hunger strike by feline companions. Anorexia or hyporexia, particularly in overnourished cats, may lead to fatty liver syndrome, which can be fatal if left untreated. In most cases of hepatic lipidosis, the animal has an underlying condition such as obesity, diabetes, neoplasia, pancreatitis or renal disease, just to name a few.

Recently, a euthanized adult male neutered cat was submitted to the VDL for autopsy. Liver failure was suspected due to widespread icterus. The history was

classic for fatty liver syndrome: an overweight cat that stopped eating and began losing weight.

Postmortem examination confirmed hepatic lipidosis. Grossly, the liver was pale to yellow with an enhanced reticular pattern. Sections of liver floated in formalin. Microscopically, nearly all hepatocytes were plump with lipid vacuoles. The examination also found evidence of chronic kidney injury, along with mineral deposition in the stomach compatible with uremia.

(Continued on page 8)



Lipid-laden hepatocytes characteristic of feline fatty liver syndrome. (Heidi Pecoraro, NDSU)

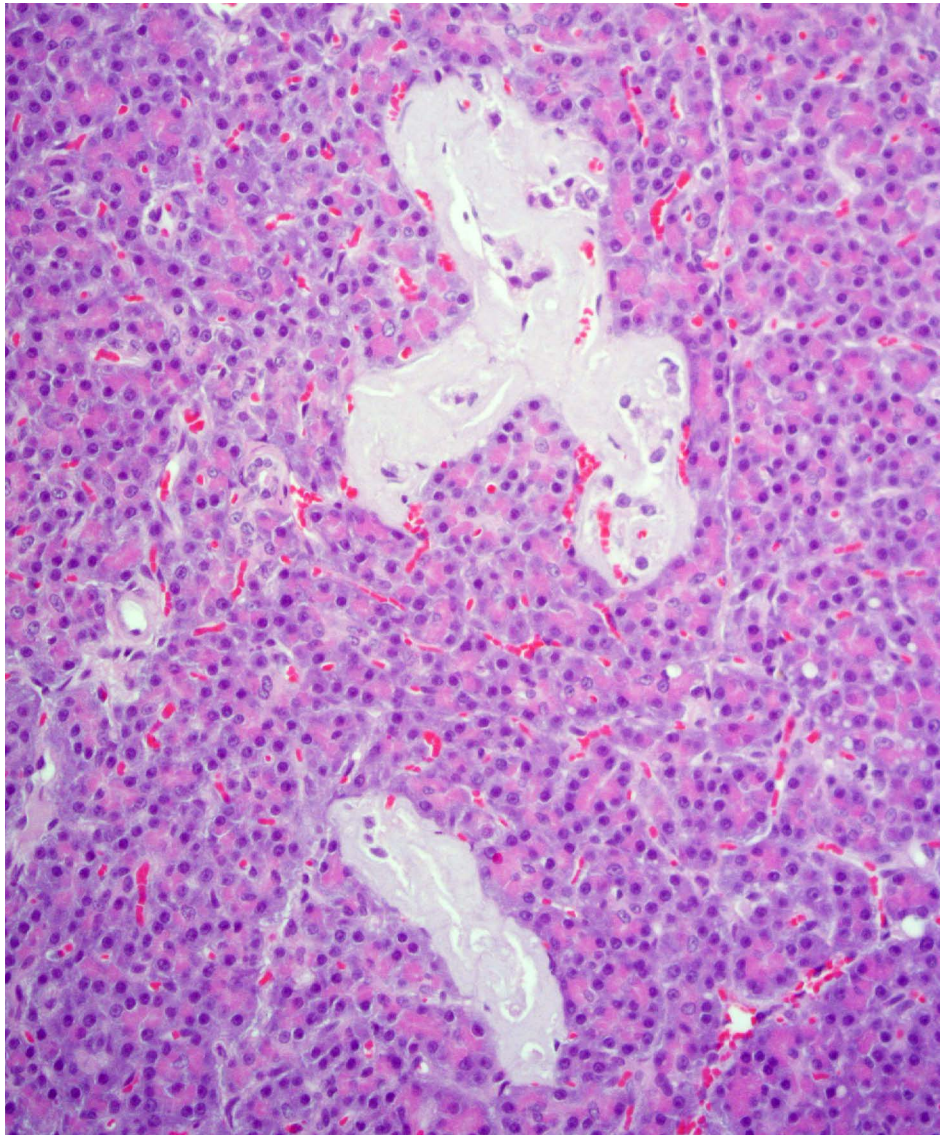
Noteworthy Cases — Feline Hepatic Lipidosis With Concurrent Renal Disease and Pancreatic Islet Amyloidosis (Continued from page 8)

Another microscopic finding was expansion of the islets of Langerhans in the pancreas by a homogenous, eosinophilic, acellular material interpreted to be amyloid. Islet amyloidosis has long been thought to be associated with diabetes mellitus in cats,¹⁻⁴ as well as in humans and macaques. However, a more recent study⁵ found that, although diabetic cats indeed have islet amyloidosis, cats without clinical diabetes also may have islet amyloidosis, and the difference between amyloid in diabetic and nondiabetic cats is not significant.

Hence, the association between islet amyloidosis and feline diabetes remains controversial. From this pathologist's perspective, islet amyloidosis may be a microscopic sign of diabetes, which is best paired with strong clinical data.

In this particular case, bloodwork was not available to determine if the cat was azotemic or hyperglycemic. However, one of the common feline diseases listed above should be considered when a cat presents with icterus and a history of inappetence.

Islets of Langerhans expanded by homogenous, eosinophilic, acellular material consistent with amyloid.
(Heidi Pecoraro, NDSU)



References

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4. Yano et al. Feline insular amyloid: association with diabetes mellitus. *Vet Pathol.* 1981;18:621-627
5. Zini et al. Endocrine pancreas in cats with diabetes mellitus. *Vet Pathol.* 2016;53(1):136-44

Contact Information

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

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