

# Reference point.

## Inside This Issue

**LINK BETWEEN DIET AND DEVELOPING DILATED CARDIOMYOPATHY IN DOGS .....1**

**A CYTOLOGY SURPRISE .....3**

**BEWARE: SAMPLE HANDLING ISSUES IMPACT COAGULATION ASSAY RESULTS .....5**

## LINK BETWEEN DIET AND DEVELOPING DILATED CARDIOMYOPATHY IN DOGS

*LAURA ANN WENNERDAHL, DVM, DIPLOMATE ACVP,  
MARSHFIELD LABS VETERINARY SERVICES*

### Dilated Cardiomyopathy

Dilated cardiomyopathy (DCM) is a disease in which cardiac musculature is abnormal, ventricular function is diminished, and there is dilation of cardiac chambers resulting in cardiac enlargement. Typically, this condition is progressive, resulting in congestive heart failure.(5) Several large and giant breeds of dogs, including Doberman Pinschers, Portuguese Water Dogs, Dalmations, Great Danes, and Boxers, are often affected.(5) Of the smaller breeds, both American and English Cocker Spaniels are predisposed. This disease is familial and inherited as an autosomal recessive or X-linked recessive trait.(5) Clinical signs include decreased energy, cough, dyspnea, and collapse.

### Atypical Dilated Cardiomyopathy

As of August 2018, the U.S Federal Drug Administration has received reports of approximately 200 suspected cases of diet-related heart disease, approximately half of which were DCM. Many of the patients were being fed “grain-free” diets (referred to as boutique, exotic, or grain-free diets) in which the predominant ingredients included potatoes or multiple legumes, their proteins, starch or derivatives and non-beef meat sources (exotic meat sources). A significant number of the affected dogs were of breeds in which familial DCM does not occur, warranting designation as “Atypical DCM”. The high potato/legume and exotic meat source dog foods were fed as the primary food source for months to years before clinical signs developed.(8)

### Taurine Deficiency

A number of dog breeds appear predisposed to developing dilated cardiomyopathy related to taurine deficiency including Golden Retrievers, Cocker Spaniels, Newfoundlands, English Setters, Saint Bernards, and Irish Wolfhounds. Certain diets were associated with taurine deficiency. In dogs, taurine is a non-essential amino acid since they can synthesize it from precursor amino acids.(6,7) Taurine is also found in dietary meat. In the body, taurine is not incorporated into protein but rather is free within many tissues and is involved in a variety of important functions.(7)

Some of the Atypical DCM dogs reported to the FDA were found to have low blood levels of taurine. Earlier studies are equivocal as to whether or not dogs could become taurine depleted from diet alone.(6) The proportions of dietary fiber and fat and composition of intestinal bacteria can alter the availability of the precursors needed to synthesize taurine.(6) It is speculated that the legumes



**Marshfield Labs™**

**BEYOND numbers**

*(Continued on page 2)*

somehow reduce the bioavailability of nutrients or interact with nutrients resulting in taurine deficiency contributing to development of DCM. Also, poor quality of protein can result in bacterial alterations and bacteria producing cholytaurine hydrolase resulting in loss of taurine from normally conserved bile acids thus contributing to taurine deficiency associated DCM.(6)

Dilated cardiomyopathy in dogs in which taurine deficiency cannot be demonstrated may also relate to diet. A preliminary study demonstrated clinical and echocardiographic improvement after diet change from certain grain free diets even when the diet was changed to another grain free diet suggesting the abnormality was not tied to the grain free status of the diet.(3)

Exotic ingredients have incompletely known nutritional and digestibility profiles and there may be other nutrient deficiencies or interactions contributing.(3) Evidence cannot determine any particular ingredients that need to be avoided, but rather the problem may be a proportion issue.(4) No recalls have been issued and no one single brand of dog food is involved.

***It is important to note that not all dogs eating these high legume diets (Boutique, Exotic, and Grain-free diets) are developing heart disease and not all dogs developing Atypical DCM have taurine deficiency.***

## Recommendations

For patients suspected of having DCM associated with grain-free/high legume diet, the following recommendations are advised:(1, 2, 3, 6)

- Record complete diet history.
- Save food, packing, and labels for future reference.
- Submit 2 mL or more of whole blood in heparin anticoagulant (green top tube) for taurine assay (University of California, Davis, Amino Acid Laboratory).
- Taurine supplementation is recommended while awaiting level results. It is inexpensive and has no reported adverse effects when administered orally.
- Change diet to a standard ingredient diet, if possible.
- Report the case to the U.S. Food and Drug Administration.

## References

1. Fascetti AJ, Reed JR, Rogers QR, Backus RC. Taurine deficiency in dogs with dilated cardiomyopathy: 12 cases (1997-2001). J Am Vet Med Assoc. 2003 Oct 15; 223(8):1137-41.
2. Freeman LM, Rush JE, Brown DJ, Roudebush P. Relationship between circulating and dietary taurine concentrations in dogs with dilated cardiomyopathy. Vet Ther. 2001 Fall; 2(4):370-8.
3. Freeman LM, Stern JA, Fries R, et al. Diet-associated dilated cardiomyopathy in dogs: what do we know? J Am Vet Med Assoc. 2018 Dec 1; 253(11):1390-1394.
4. Lau E. Unconventional dog foods suspected in heart disease. VIN news service, Sept. 4, 2018.
5. Miller L, Van Vleet J, and Gal A. Cardiovascular system. In: Zachary J, ed. Pathological Basis of Veterinary Disease. 5th ed. Elsevier; 2012:583.
6. Sanderson SL. Taurine and carnitine in canine cardiomyopathy. Vet Clin North Am Small Anim Pract. 2006 Nov;36(6):1325-43, vii-viii.
7. Torres CL, Backus RC, Fascetti AJ, and Rogers QR. Taurine status in normal dogs fed a commercial diet associated with taurine deficiency and dilated cardiomyopathy. J. Anim. Physiol. a. Anim. Nutr. 87(2003):359-372.
8. U.S. Federal Drug Administration. FDA investigating potential connection between diet and cases of canine heart disease. July 12, 2018.

(Continued on page 3)

# A CYTOLOGY SURPRISE

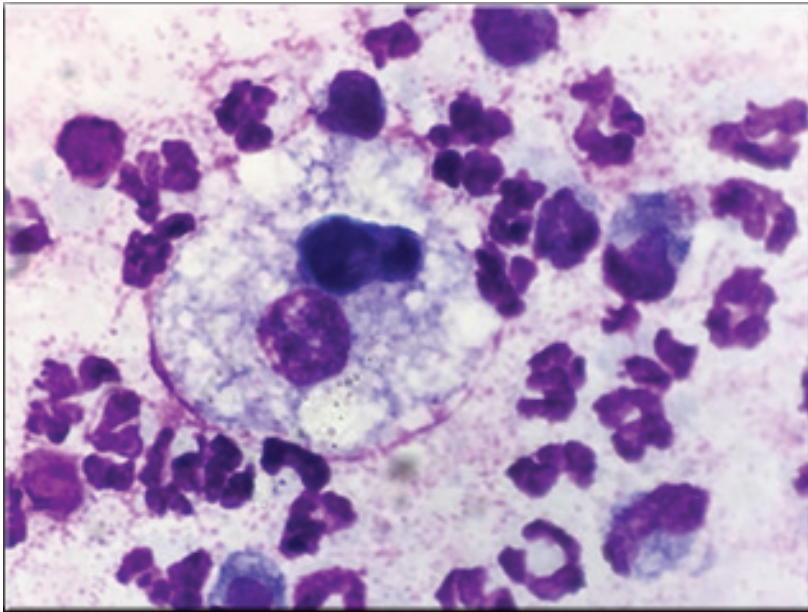
MATTHEW FEIRER, DVM, DIPLOMATE ACVP & LAURA ANN WENNERDAHL, DVM, DIPLOMATE ACVP,  
MARSHFIELD LABS VETERINARY SERVICES

Submitted for cytological examination is a tracheal wash from a 5 year old, male, neutered, German Shepherd dog. No clinical history was provided. Slides and white topped and EDTA/lavender topped tubes containing tracheal wash fluid were submitted.

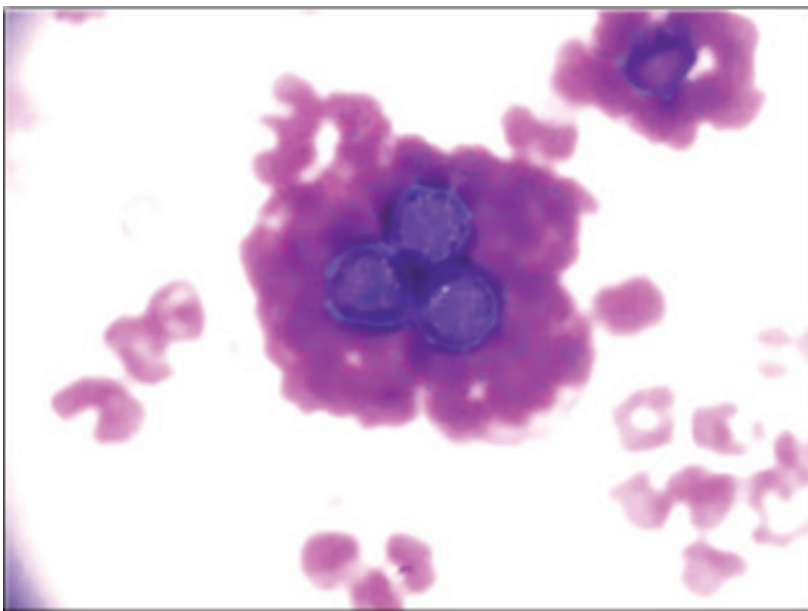
## Cytological Findings

### EDTA tube smears:

Slides were of excellent quality, and contained a marked, pyogranulomatous infiltrate. Admixed throughout were moderate to large numbers of often broad based budding fungal yeast, which were morphologically consistent with *Blastomyces dermatitidis*. They were observed both free in the background and occasionally phagocytosed within macrophages.



*Numerous neutrophils surround a large foamy macrophage containing two yeast organisms.*



*Three fungal yeast forms amid red blood cells.*

(Continued on page 4)

### **White top tube smears:**

In addition, on the slides prepared from the white topped tube only, many occasionally septate, and thin hyphae are seen extending from the yeast.

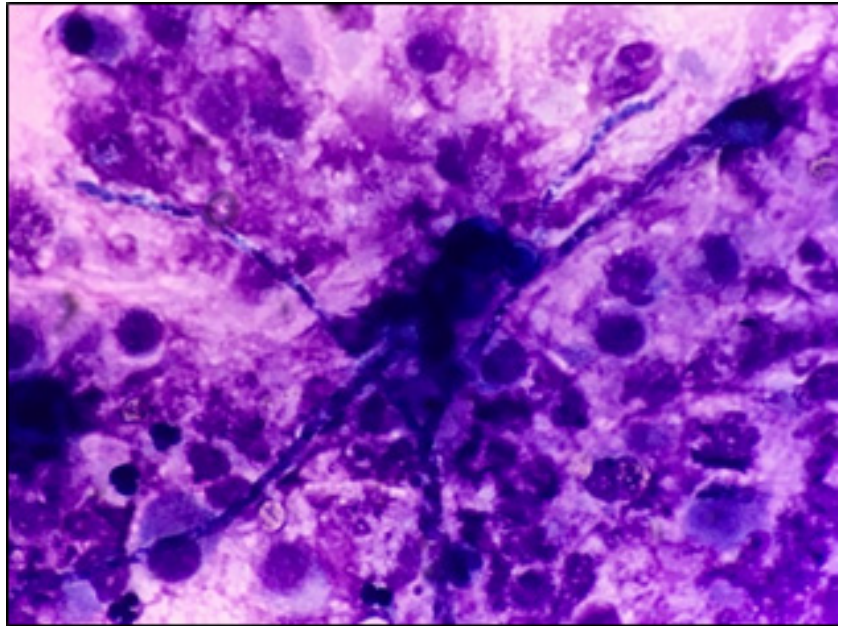
This is a rare finding on cytology, as hyphae are rarely seen with *Blastomyces* infections. They are consistent with the mycelial form. In this instance, they most likely started to grow in transit to the lab. White topped tubes are permissive for microbial growth, and lavender topped EDTA tubes are not, which in this case explains the presence of hyphae in the white topped tube only.

**Diagnosis:** *Blastomyces dermatitidis*

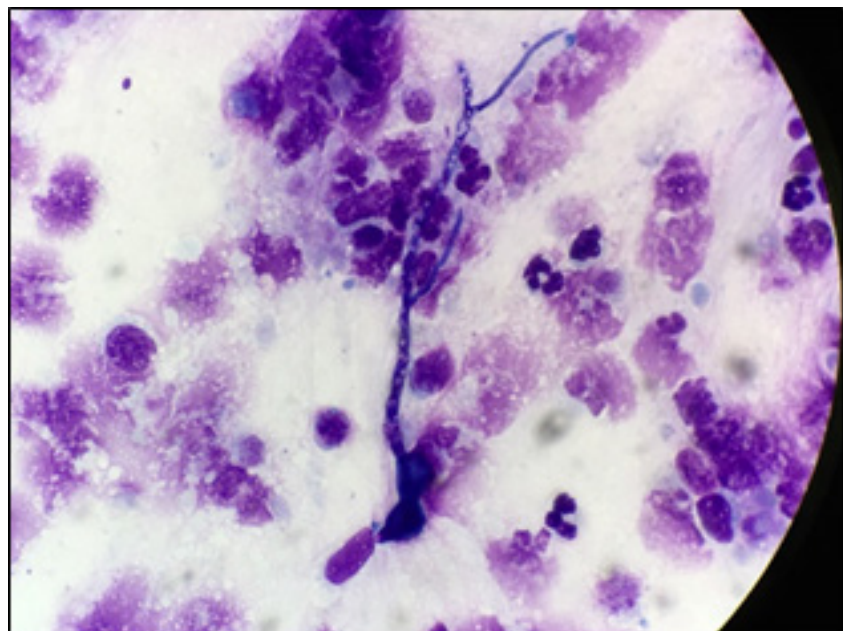
### **Summary**

*Blastomyces dermatitidis* is a dimorphic soil saprophyte, which is endemic in wet, swampy areas including Mississippi and Ohio River valleys. Dogs are infected more commonly than cats. Clinical signs depend on the tissues infected, with the respiratory system most commonly affected. Ocular infection occurs in approximately 50% of dogs with systemic Blastomycosis. Other common sites of involvement include the skin, bone, and joints.

*Blastomyces* infection causes pronounced pyogranulomatous to granulomatous inflammation. In tissues, the organisms are observed in the yeast form. Yeast forms are round to oval, 5 to 25 um in diameter with thick, sharply defined, refractile cell walls. In soil, the organisms are in the mycelial form (*Ajellomyces dermatitidis*) which reproduces asexually with small conidia (asexual spores). The conidia are the main infectious particles which when inhaled, result in pulmonary infection.



*Long thin mycelial hyphae extend from a yeast form.*



*Multiple long thin mycelial hyphae extend from yeast forms.*

(Continued on page 5)

# BEWARE: SAMPLE HANDLING ISSUES IMPACT COAGULATION ASSAY RESULTS

FRANCES MOORE, DVM, DIPLOMATE ACVP, MARSHFIELD LABS VETERINARY SERVICES

Coagulation assays [prothrombin time (PT), activated partial thromboplastin time (aPTT) and fibrinogen] are performed on some of the most critically ill patients. Conditions during the process of blood draw and handling of the sample prior to submission to the laboratory can have a crucial impact on the results of these important assays.

Difficulty in locating the vein, sample hemolysis, inappropriate blood volume to anticoagulant ratio in the tube, and failure to render samples platelet poor prior to freezing can invalidate coagulation results. Excessive trauma during search for a vein and sample hemolysis can activate the coagulation cascade, shortening the results of coagulation assays. Underfilling citrate tubes causes overcoagulation of the sample and can prolong coagulation assay results. Freezing plasma which has not been rendered platelet poor by adequate centrifugation releases platelet phospholipids into the plasma, activating coagulation and resulting in artificially shortened coagulation assays.

Guidelines from the Clinical and Laboratory Standards Institute for collecting, transporting, and storing blood for coagulation testing recommend centrifugation of citrated plasma for 15 minutes at 1500 g to obtain a sample with platelet count <10,000/uL prior to performance of coagulation tests. Similarly, samples must be rendered platelet poor prior to freezing of the plasma (which will release platelet phospholipids).

Optimum blood drawing techniques and attention to sample handling variables can ensure results appropriately reflect the coagulation status in these critically ill patients.

## References

1. Magnette A, Chatelain M, Chatelain B, TenCate H, Mullier F. Pre-analytical issues in the haemostasis laboratory: guidance for the clinical laboratories. *Thrombosis Journal* 2016; 14:49.
2. Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline - Fifth Edition. Clinical and Laboratory Standards Institute H21-A5; Vol 28; No 5.

