Kansas State Veterinary
DIAGNOSTIC LABORATORY

DIAGNOSTIC *INSIGHTS*

JANUARY 2018

Toxoplasma Gondii Causing Abortion in a Goat

By Dr. Sarah Schneider

Background: *Toxoplasma gondii* is an important cause of abortion in sheep and goats. *Toxoplasma gondii* is a protozoal organism whose infective oocysts are shed in the feces of infected cats, which are the definitive host. Animals become infected by ingesting oocysts in contaminated feed or soil. Abortion and neonatal mortality occur if sheep and goats are first infected during early to mid-pregnancy.

History: Abortions were occurring in a well-vaccinated goat herd. A well-preserved male Boer goat fetus and a tube of abomasal contents were submitted.

Diagnostics: Necropsy was performed on an approximately 12-week well-preserved fetus with no gross lesions (figure 1). Minimal postmortem autolysis suggests the kid was alive or died just before it was aborted. Histology showed multifocal necrosis and non-suppurative inflammation in multiple tissues including brain, heart, kidney and skeletal muscle (Brain: multifocal necrosis and non-suppurative encephalitis [figure 2]). Aerobic, *Brucella* and *Campylobacter* culture were negative on both the fetal abomasal fluid and the submitted sample of abomasal fluid. Leptospira PCR, Chlamydia PCR, and virus isolation on pooled fetal tissues were negative. Heart blood was positive for Toxoplasma antibody by indirect ELISA.

Diagnosis: Abortion due to Toxoplasma gondii.

Outcome: The dam rarely shows clinical signs of infection, and in healthy animals only the initial infection will cause an abortion, meaning that once seroconversion occurs in the dam, the long-term prognosis for the dam and future pregnancies is good.

Take home message: Toxoplasmosis is an important cause of abortions, particularly in young does. As in this case, the fetus often has few or no lesions, and so placenta is the preferred sample when toxoplasmosis is suspected in small ruminants. The lesions are most evident in the placenta, while brain and skeletal muscle are the second and third most likely tissues to be positive. Reducing the risk of abortion in goats due to toxoplasmosis involves exposing new breeding

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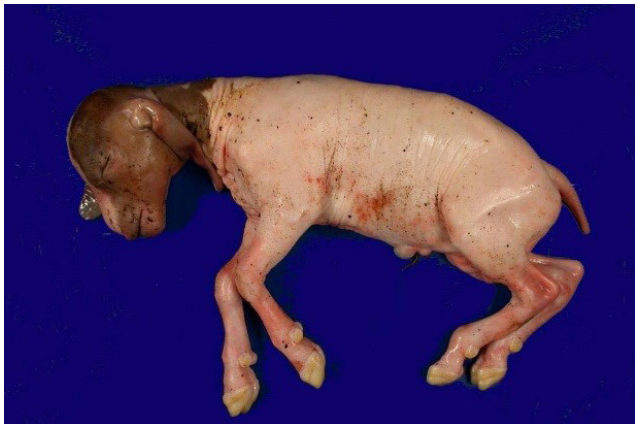

Figure 1

In this Issue

Accredited by the American Association of Veterinary Laboratory Diagnosticians

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www.ksvdl.org/accounting-and-billing/

Cutaneous Epitheliotrophic Lymphoma/Mycosis Fungoides in Dogs:
Clinical and Histopathological Characteristics

By: Dr. Charan Ganta

Cutaneous Epitheliotrophic Lymphoma in Dogs

Cutaneous epitheliotrophic lymphoma is a rare but progressive disease of older dogs with average age of 8-10 years. The disease is characterized by infiltrations of neoplastic T lymphocytes that show tropism towards epidermis and adnexal structures. The average time from first onset of clinical signs to diagnosis of the disease is 5.5 months. There is some breed predisposition in English cocker spaniels and boxers, however other breeds could be affected.

Clinical Presentation of Epitheliotrophic Lymphoma

Clinical presentation of this condition is non-specific and can mimic many skin conditions. It affects skin and mucocutaneous junctions or oral mucosa. The clinical presentation is highly variable and hence it is classified into 4 different categories (Figure 1).

1. Exfoliative erythroderma: Characterized by erythema over large areas of the body with scaling, depigmentation and alopecia; often these lesions will progress to plaques, patches and nodules. The distribution of lesions is generalized but most frequently involves head and trunk.

2. Mucocutaneous Form: This form is common in dogs and often affects lips, nose and eyelids. The lesions are characterized by erythema, depigmentation, alopecia, irregular infiltration, erosion, and ulceration. Depigmentation is a common and clinically striking feature with marked bilateral symmetry.

3. Solitary or multifocal cutaneous plaques or nodules: Characterized by solitary or multiple plaques or nodules that usually are erythematous and scaly or crusted. The larger lesions often erode and ulcerate. This stage can progress to neoplastic lymphadenopathy with eventual systemic spread.

4. Ulcerative disease of oral mucosa: Oral mucosal involvement is not uncommon in dogs and can sometimes be limited to the oral mucosa; commonly affects gingiva, palate, or tongue that can progress to ulcerations.

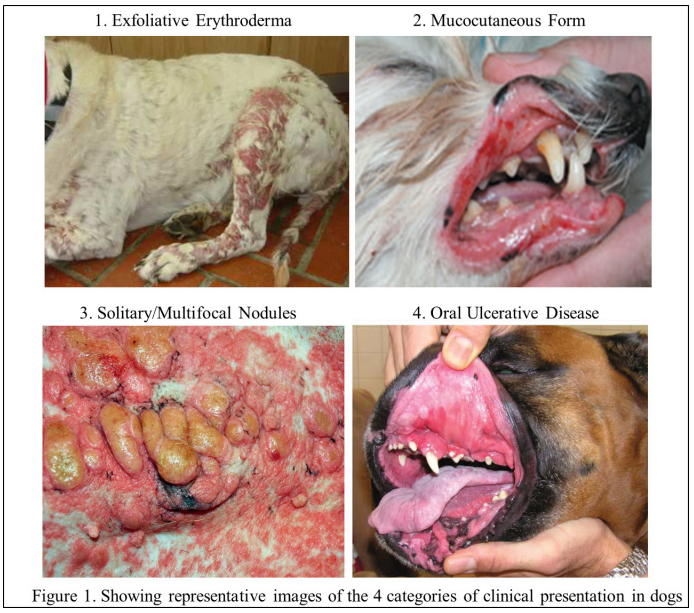

Figure 1. Showing representative images of the 4 categories of clinical presentation in dogs

Biopsy Sample Collection and Histopathology

Epitheliotrophism cannot be identified by cytology - a definitive diagnosis is always based on histopathological examination. Hence, a full-thickness skin biopsy section is required for a definitive diagnosis. A characteristic infiltration of lymphocytes along the lower layers of the epidermis or mucosal epithelium and adnexal structures including the hair follicular walls and apocrine glands is considered diagnostic (Figure 2).

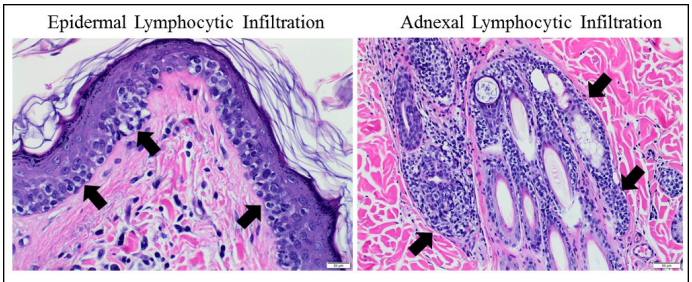

Figure 2. H&E sections of skin biopsy. Arrows showing lymphocytic infiltrations into the lower layers of the epidermis

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Antigen Blocking and Heartworm Heat Treatment Antigen ELISA

By Dr. Brian Herrin

The most common platform for testing dogs and cats for heartworm is an antigen ELISA or lateral flow assay (e.g. IDEXX SNAP® 4Dx®Plus/Heartworm, Heska SoloStep®, Zoetis Witness™ and DiroCHEK®, Abaxis VetScan®, etc.). These tests all detect a similar analyte, a uterine antigen produced predominantly by mature, female heartworms. While there are several reasons for false negative test results, including low numbers of worms, immature worms, or only male worms, the veterinary community is currently learning much more on a phenomenon known as antigen blocking. Antigen blocking occurs when antigen-antibody complexes form in the blood, binding up the circulating antigen and preventing it from reacting with the diagnostic kit. (FIGURE 1-A; FIGURE 2) Since the antibodies are binding the heartworm antigen within the blood, using a different antigen test will not alleviate the problem. This phenomenon has been documented in all current heartworm antigen test platforms.

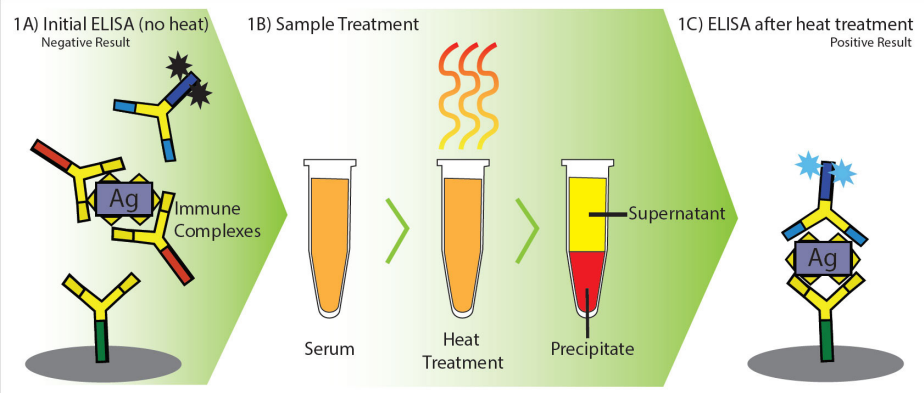

Figure 1. Schematic depiction of A) antibody-antigen complexes preventing the detection of heartworm antigen, B) heat treating serum to dissociate the complexes, and C) the resulting positive test once the antibody has been removed. (Modified from Beall MJ, et al. 20172)

What can we do about it?

After looking through the literature, researchers found that the first heartworm antigen kits came with an antibody dissociation step, which was removed in the newer test platforms[1]. This step used an acid to break up the antibody, releasing the antigen to be detected by the kit[2] (FIGURE 1-C; FIGURE 2). We now know this can be done with a heat step as well (FIGURE 1-B), and many labs, including KSVDL, now offer this test as a supportive diagnostic in working up animals with clinical signs consistent with heartworm disease.

Currently the American Heartworm Society (AHS), Companion Animal Parasite Council (CAPC), and veterinary diagnostic labs DO NOT promote the use of a heat treatment step for every patient. In fact, degrading the antibodies would invalidate kits that detect antibodies to tick-borne diseases or other agents. Instead, veterinarians can submit at least 1 mL of serum to KSVDL and request the "Heartworm Heat Treatment Antigen ELISA."

Who does this affect?

Since the exact cause is not known, veterinarians are unable to predict which animals may experience this Ag-Ab complexing, but it clearly involves an immune response and therefore patients with inflammatory conditions may be more likely to develop blocking antibodies. The current literature has described several common scenarios where antigen blocking may be routinely seen. After heat-treating negative heartworm antigen samples, approximately 5-10% of shelter dogs[3] and 6-8% of shelter and feral cats[4] reversed to a true positive test result. While these animals do not represent well-cared-for pets, which have a much lower incidence of this phenomenon, they are often adopted out to owners who may

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Vitamin A and E Shortage and the Potential Impact on Cow-Calf herds

By Drs. Gregg A. Hanzlicek and Steve Ensley

There is a worldwide Vitamin A and E shortage presently occurring, and the situation is expected to continue for some time. As a consequence, cattle mineral costs are expected to increase significantly.

Vitamin E is very important to the bovine immune system. Research suggests that deficiencies in dietary Vitamin E can result in increased disease incidence including mastitis, metritis with or without retained placentas, and increased days from calving to conception[1].

Over the last five years, the KSVDL has diagnosed late term weak calves and stillborn issues related to dietary Vitamin A deficiency in 5 - 20 different cow-calf herds, depending on the year.

To better assure cow and calf health, it is important that producers not reduce the dietary supplementation of these two important elements in an attempt to reduce feed costs during the shortage period.

If you suspect a deficiency of either of these two vitamins in herds experiencing health issues or stillborn and weak calves, excellent diagnostic tests are available.

The preferred post-mortem tissue is liver, and the preferred ante-mortem sample is serum. Vitamin E can be analyzed on whole blood. One ml of serum for vitamin A & E or 1 ml of whole blood for vitamin E is sufficient.

For more information contact KSVDL Client Care at 866-512-5650 or clientcare@vet.k-state.edu.

[1] Nutrient Requirements of Beef Cattle. Eight Revised Edition. National Academies Press. Washington, DC. 2016; page 260.

Meet Dr. Steve Ensley

Dr. Steve Ensley graduated in 1981 from Kansas State University with a DVM. After 14 years in mixed practice in the midwest he received a MS and PhD in veterinary toxicology at Iowa State University completing his PhD in 2000. Dr. Ensley has worked for the University of Nebraska-Lincoln as an assistant professor and veterinary toxicologist. Dr. Ensley has also worked for Bayer AG in Kansas City before returning to Iowa State University working in the Department of Veterinary Diagnostic and Production Animal Medicine (VDPAM) in 2006. In November 2017 Dr. Ensley moved to Kansas State University and works as a clinical veterinary toxicologist.

Dr. Ensley's interests are clinical veterinary toxicology and applied veterinary toxicology research. Dr Ensley has published extensively on applied veterinary toxicology and gives numerous presentations on these topics. Food animal veterinary toxicology is his passion.

HEARTWORM *(continued from page 3)*

rightly believe they have a heartworm negative animal. In addition, 54% of dogs[5] who are put on the "slow-kill" heartworm treatment had the same reversal to a positive test result. This may lead veterinarians to believe the "slow-kill" method is more effective or rapid than it actually is, which is why the AHS still does not recommend this as a true treatment option.

Take-home points

This can happen with ANY heartworm antigen test kit, regardless of manufacturer

Sample heating should not be performed in general practices at this time

Occurs more frequently in animals with no or sporadic history of heartworm preventive

Serum heat-treatment should only be performed for animals with a clinical history and signs supportive of heartworm disease in the face of a negative antigen test

A minimum of 1 mL of serum is needed to run the test

If you have any challenging heartworm cases or questions about when to request the "Heartworm Heat Treatment Antigen ELISA" please contact

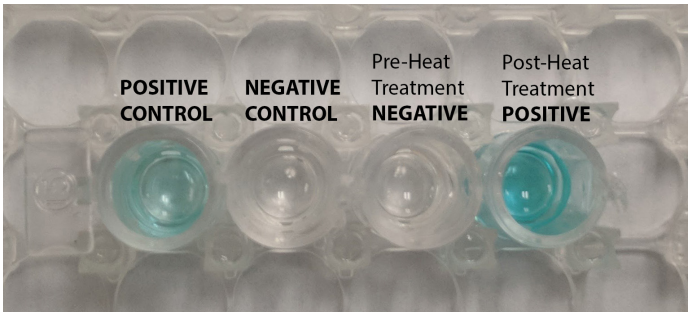

Figure 2. Picture of a commercially available, well-based antigen ELISA. This photo shows the result of a serum sample before and after heat-treatment. After heat treatment, the antigen is free to interact with the diagnostic kit, which results in a positive test.

Dr. Brian Herrin at the KSVDL (785-532-4430) or KSVDL Client Care (785-512-5650).

References

1 Little SE, et al., Pre-treatment with heat facilitates detection of antigen of Dirofilaria immitis in canine samples. Vet Parasit. 2014;203:250-252.

2 Beall MJ, et al. Validation of immune complex dissociation methods for use with heartworm antigen tests. Parasit Vector. 2017;10(Suppl 2):481.

3 Velasquez L, et al. Increased prevalence of Dirofilaria immitis antigen in canine samples after heat treatment. Vet Parasitol. 2014;206(1-2):67-70.

4 Gruntmeir JM, et al. Increased detection of Dirofilaria immitis antigen in cats after heat pretreatment of samples. J Feline Med Surg. 2017;19(10):1013-1016.

5 Drake J, et al., False negative antigen tests in dogs infected with heartworm and placed on macrocyclic lactone preventives. 2015;8:68.

TOXOPLASMA GONDII (cont'd from page 1)

animals to the herd early to allow them time to seroconvert, and reducing exposure to Toxoplasma oocysts, by limiting the access of barn cats to areas of goat feeding, and spaying barn cats (oocysts are most likely to be passed by young, newly infected cats.)

References:

David Buxton. Protozoan infections (Toxoplasma gondii, Neospora caninum and Sarcocystis spp.) in sheep and goats: recent advances. Veterinary Research, BioMed Central, 1998, 29 (3-4), pp.289-310

LYMPHOMA *(cont'd from page 2)*

Prognosis:
The prognosis is often poor and is dependent on the clinical stage of presentation. The average age of survival is a few months to 2.5 years from the time of diagnosis. For more information on available treatment options please contact an oncologist at the Veterinary Health Center (VHC), College of Veterinary Medicine, Kansas State University. Please call KSVDL Client Care (866-512-5650) to setup an oncology consult with one of the oncologist at VHC.

Follow this link for "6 Tips for Biopsy Submissions"

https://www.youtube.com/watch?v=79v69x7qLFs

KSVDL Personnel Activities

Activities

Dr. Lalitha Peddireddi was a faculty volunteer for interviewing prospective 2022 CVM students.
Dr. Steve Ensley conducted a liver biopsy wet lab at the North Dakota Veterinary Medical Association in Fargo, ND.
Dr. Doug Marthaler Attended the American Association of American Veterinary Laboratory Diagnosticians and co-organizing the symposium entitled, "Next Generation Sequencing: Application in Veterinary Diagnostic Laboratories, A Multidisciplinary Symposium". He also Co-chaired the Virology Committee in San Diego, CA.
Dr. Jianfa Bai presented a poster at the Kansas Veterinary Medical Association Conference in Manhattan, KS.
Dr. Steve Ensley conducted a liver biopsy wet lab at the Colorado Veterinary Medical Association Continuing Education Ag Animal Program in Greeley, Co.
Dr. Lalitha Peddireddi presented, "Development of a quantitative real time RT-PCR assay for sensitive detection of emerging Atypical Porcine Pestivirus associated with congenital tremor in pigs" at the CRWAD Annual meeting in Chicago, IL.
Dr. Cindy Bell presented a poster at the Kansas Veterinary Medical Association Conference in Manhattan, KS.
Drs. Mike Moore and Gregg Hanzlicek represented the KSVDL at the Kansas Veterinary Medical Association Conference in Manhattan, KS.
Dr. Doug Marthaler presented "Molecular epidemiology, clinical relevance and diagnosis of porcine rotaviruses A, B, and C" at the Western Canadian Association of Swine Veterinarians in Saskatoon, SK.
Dr. Lalitha Peddireddi was awarded a grant as co-principle investigator with Dr. Kenneth Harken, VHC, titled: An evaluation of the persistence of shedding of pathogenic leptospires in the urine of dogs with leptospirosis by polymerase chain reaction assay.
Dr. Doug Marthaler presented a poster at the Kansas Veterinary Medical Association Conference in Manhattan, KS.
Dr. Lalitha Peddireddi served as a moderator for the Companion Animal Epidemiology session at the CRWAD meeting in Chicago, IL.
Dr. Gregg Hanzlicek lead a discussion on Anaplasmosis at the Bern-Sabetha Veterinary Clinic's client appreciation banquet in Bern, KS.

Field Investigations

Respiratory issues in milk-fed calves housed in an environmentally controlled environment
Chronic low milk production on a Kansas dairy
Johne's risk assessment exercise on a Kansas cow-calf operation

Developing and Delivering Accurate, Innovative Diagnostic Services

The mission of the Kansas State Veterinary Diagnostic Laboratory (KSVDL) is to develop and deliver accurate, innovative, and timely diagnostic and consultative services to the veterinary and animal health community while providing support for teaching, training and research programs.

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Continuing Education

www.vet.k-state.edu/education/continuing/

January 25-27, 2018
Oklahoma 103rd Annual Veterinary Medical Association Conference
Oklahoma City, Oklahoma
https://okvma.org/ovma-annual-convention/

February 16, 2018
Cow-Calf Conference
"Use of Information Technology to Enhance Veterinary Services for Cow-Calf Herds"
Stanley Stout Building
Manhattan, Kansas
http://www.vet.k-state.edu/education/continuing/conferences/cowcalf-conf18/

February 25, 2018
35th Annual Frank Jordan Conference
"Use of Information Technology to Enhance Veterinary Services for Cow-Calf Herds"
Frick Auditorium
Kansas State College of Veterinary Medicine
Manhattan, Kansas
http://www.vet.k-state.edu/education/continuing/conferences/FWJ18/index.html

March 4-6, 2018
Western States Veterinary Conference
Las Vegas, Nevada
https://www.wvc.org/

For more information, call the Continuing Education Office at 785-532-4528.

Test Results and Schedules

Laboratory results available online, all the time!

Holiday Schedule:

Memorial Day: Closed Monday, May 28th
Open Saturday, May 26th , normal business hours (8 a.m. to 12 noon)
Open Tuesday, May 29th, normal business hours

To receive this newsletter by email, contact: ksvdloutreach@vet.k-state.edu.

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