

Diagnostic Insights

Kansas State Veterinary Diagnostic Laboratory
www.ksvdl.org



Accredited by the American Association of Veterinary Laboratory Diagnosticians

September 2013

KSVDL Canine Brucellosis Testing Options

Canine Brucellosis remains an important infectious disease in Kansas and the Midwest. Various testing strategies are an integral part of the diagnosing and the prevention of the disease. In addition, specific and timely testing is also required for dogs export to selected countries. Canine brucellosis is a contagious disease in dogs caused by *Brucella canis*, an intracellular gram negative organism. While many infected dogs are asymptomatic, clinical signs in males include infertility, epididymitis, orchitis, testicular atrophy, scrotal dermatitis and/or diskospondylitis. Those female dogs with overt signs may show infertilities or fetal abortions.

1. Canine Brucellosis Culture (Microbiology Laboratory)

While a canine Brucellosis culture is not as sensitive as serology antibody test, a positive *Brucella canis* cultures is considered a definitive diagnosis. A sterile blood culture is the most common sampling method for culturing Brucellosis. However, a negative blood culture does not rule out the disease since in some dogs, the required bacteremia is intermittent. This will result in a possible "false negative" blood culture. Recent antibacterial therapy may also temporarily inhibit the bacteremia resulting in a negatively culture. In addition, any bacterial contamination of the blood sample during collection will quickly overgrow the blood sample during incubation making *B. canis* impossible to identify.

Samples: Sterile whole blood culture in sodium citrate tubes, infected tissue or aborted/still-born puppy.

Cost \$3.00 and requires 4+ days of incubation.

For more information contact Dr. Brian Lubbers (785-532-4012 or blubbers@vet.k-state.edu)

2. Canine Brucellosis Real-Time PCR Test (Molecular Diagnostics Laboratory)

A real-time PCR test to identify *Brucella canis* is available at the KSVDL. The test is a duplex real-time PCR procedure targeting the 16S rRNA gene that is common to all *Brucella* species, and a DNA fragment that is specific to *Brucella canis*. This test is more sensitive than a blood culture.

Samples: Sterile blood culture in sodium citrate tubes or vaginal swabs in a sterile tube are the preferred sample types. The cost for the first 10 samples is \$30.00 each, and for more than 11 samples the cost is \$25.00 each.

For more information contact Dr. Jianfa Bai (785-532-4332 or jbai@vet.ksu.edu)

3. Canine Brucellosis ME Tube Agglutination Test (ME-TAT) (Serology Laboratory)

The highly sensitive ME TAT agglutination screening test detects *Brucellosis canis* antibodies. This is the same test used by the National Veterinary Services Laboratories (NVSL) in Ames, IA and is an approved for exporting most dogs or semen.

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To set up an account go to:
www.ksvdl.org

Certifact[®]-triggered pemphigus foliaceus in dogs

Dr. Gordon Andrews

Pemphigus foliaceus (PF) is an autoimmune skin disease of dogs, humans, and several other animal species. The lesions of PF are fragile subcorneal pustules that progress to erosions and crusts. In dogs, skin lesions are typically observed on the face, nasal planum, ears, and paw pads. The lesions can be localized or involve the entire body.

An autoimmune pathogenesis for most cases of canine PF is supported by the demonstration of circulating antikeratinocyte IgG autoantibodies directed against molecules of the desmosomes between cells. The diagnostic microscopic feature is the presence of acantholytic keratinocytes within the subcorneal pustules. Acantholytic keratinocytes are individualized rounded-up cells of the stratum spinosum and present either singly or in “rafts” resulting from loss of cohesion between epithelial cells.

I recently received skin punch biopsies from an 11 year old spayed female Labrador Retriever. No clinical history was provided on the submission form except for “suspect pemphigus foliaceus”. The original histology slides I examined were non-specific hyperplastic dermatitis, so I contacted the submitting veterinarian for additional clinical information. In the meantime, I requested additional slides be cut deeper into the tissue.

The clinical history indicated that topical Certifact[®] had been applied to the skin between the shoulder blades. The patient subsequently developed an area of alopecia, thickened, scaly, and scabby skin at the site of application which was spreading along the dorsal midline. The biopsies were from this area, but the patient was also described as having scabby lesions between the toes. Photographs of the patient were also provided. (Figure 1) Examination of the additional microscopic slides revealed a few areas of subcorneal pustular dermatitis with acantholytic keratinocytes diagnostic of pemphigus foliaceus. (Figure 2)

Certifact[®] (Merial, Duluth GA, USA) is a topical flea, tick, and mite control product that contains fipronil, (s)-methoprene (an insect growth regulator), and amitraz, and has been documented to trigger the development of an acantholytic pustular dermatitis similar to that

of Promeris[®]-triggered PF and naturally occurring autoimmune pemphigus foliaceus (AIPF) in dogs. Promeris

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Figure 1: Alopecia with scales and crusts between the shoulders and extending caudally which developed following topical application of Certifact.

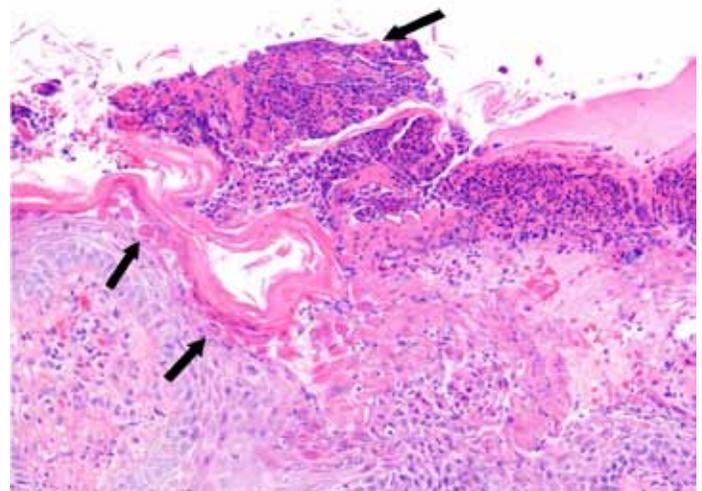


Figure 2: Photomicrograph of the skin showing subcorneal neutrophilic pustule containing acantholytic keratinocytes (arrows).



FOLIACEUS | continued from Page 2

Duo® (Pfizer, New York, NY, USA) is another topical antiparasitic drug combination for flea and tick control in dogs that contains metaflumizone and amitraz. Production of Promeris® was stopped in 2011 following publication of evidence linking the product with the development of PF in dogs. Clinically, in both Certifect® and Promeris® triggered PF, dogs affected tended to be middle-aged to older, female, large-breed dogs, including Labrador Retrievers as in the present case.

Skin lesions develop after as few as one application, or after 7 or 8 doses. Additionally, lesions develop in as few as 14 days, but they may not develop until several months following application. Approximately 33% of the dogs have skin lesions localized to the site of application, with the remainder also having skin lesions at distant, non-contiguous sites. Distant sites included pinna, face, footpads, trunk and ventrum, interdigital skin, and nasal planum. Those with distant lesions also tend to have systemic signs including lethargy, lameness, fever, anorexia, and pain. Systemic signs are not confined only to those with distant lesions. Dogs with localized lesions tend to respond more favorably to immunosuppressive therapy compared to those with distant lesions, and those with distant lesions more frequently require combination immunosuppressive therapy and longer treatment duration. Histopathological findings in Certifect®-triggered PF, Promeris®-triggered PF, and AIPF are identical.

It is unknown if individual active drugs in these parasite control products, ingredients in the product vehicle, or some combination of ingredients are responsible for triggering PF in dogs. Diagnosis is based on characteristic histopathological lesions and history of drug application. Multiple formalin-fixed skin biopsies of intact pustules and a thorough clinical history are essential in establishing a definitive diagnosis.

References:

Certifect-triggered pemphigus foliaceus in dogs: clinical, histological and immunological characteristics. P.Bizikova, K.Linder and T.Olivry. Abstracts of the 26th Annual Congress of the ECVD-ESVD, 19-21 September 2013, Valencia, Spain. Vet Dermatol 2013;24:378

Metaflumizone–amitraz (Promeris)-associated pustular acantholytic dermatitis in 22 dogs: evidence suggests contact drug-triggered pemphigus foliaceus. U.Oberkirchner, K.E.Linder, S.Dunston, P.Bizikova and T.Olivry. Vet Dermatol 2011;22:436-448

3rd Annual KSVDL Conference on Animal Diagnostics

The KSVDL will be holding our 3rd annual continuing program on Saturday, Feb. 8, 2014, from 9 a.m. to 3:30 p.m.

Program focus:

Bovine Vaccinology

Guest speaker:

Dr. Amelia Woolums
University of Georgia
College of Veterinary Medicine

Topics presented will include:

What's new about:

- bovine immunology
- vaccine timing
- modified live vs. killed product use
- how to read a titer
- autogenous vaccines

Stay tuned for more information about this timely continuing education program.



New Canine and Feline Mycoplasma PCR Test

Haemoplasmas are haemotropic mycoplasmas and are associated with anemia. A PCR test to identify two haemoplasmas in both canine and feline blood samples is now available at the Kansas State Veterinary Diagnostic Laboratory. This test offers a great advantage of specific and rapid detection of canine and feline haemoplasmas organisms.

In canine blood samples, the PCR detects both *Mycoplasma haemocanis* and *Mycoplasma haemoparvum*.

In feline blood samples, the PCR detects *Mycoplasma haemofelis* and *Mycoplasma haemominutum*.

The KSVDL is requesting some additional samples to further validate this test: Unclothed blood (purple-top tube) from confirmed (treated and untreated) cases is the preferred sample. We would also like samples from: 1) suspected positive animals showing clinical signs, and 2) immunocompromised animals with symptoms of anemia.

Sample: 2 ml; unclotted blood (purple top tube);

Shipping: On ice (shipped overnight if necessary)

Cost: Free for validation testing

Turnaround time: 48 hours after the receipt of the samples

Contact: Dr. Lalitha Peddireddi at 785-532-5651 or Lalitha@vet.ksu.edu for more information.

Camelid IgG Test Validated

A recent collaborative effort between Dr. Maria Ferrer, Clinical Associate Professor, Theriogenology, and Dr. Melinda Wilkerson, Professor, Immunology, has led to the internal validation of a radial immunodiffusion (RID) immunoglobulin G (IgG) test. Radial immunodiffusion is the most accurate measurement technique for IgG.

This test is an important tool to determine the passive transfer status of crias, and it can also be used as to screen adult animals suspected of being immunodeficient.

The test results will be reported with both a quantitative value (adequate or inadequate passive transfer) and a qualitative measurement of actual IgG levels.

To test for passive transfer, one blood sample needs to be collected at 24-48 of age.

Sample: 1ml serum (red-top tube)

Estimated Result Time: within 24 hours of receipt of the sample (except on Fridays)

Cost: \$39/sample

Contact person: Melinda Wilkerson @ 785-532-4818 or wilkerns@vet.k-state.edu.

BRUCELLOSIS | continued from Page 1

Samples: 3 mls of serum: Cost: \$8.20

Schedule: Monday and Wednesday with results in 1-2 business days

For more information contact Dr. William Fortney (783-532-4605 or wfortney@vet.keu.edu)

Note: Canine Brucellosis is a reportable disease in Kansas, so all positive tests run at the Kansas State Veterinary Diagnostic

Laboratory or any laboratory on animal from Kansas are to be reported to the Kansas Division of Animal Health at 785-296-2326.

For more information on all our Canine Brucellosis Tests, Fees, Schedules, and Sample Submissions, please visit our website at www.ksvdl.org or contact Client Care at clientcare@vet.k-state.edu or 785-532-4349



Hot Topic: Tularemia

Dr. Ram Raghavan and
Dr. Brad DeBey

Tularemia, an occasional disease of cats in the Midwestern U.S. is highly fatal and a serious public health concern due to its zoonotic potential. Humans can acquire this disease from contact with cats but also from biting insects (flies, ticks) that have fed on infected animals. Another common infection source is accidental inhalation of the bacteria (*Francisella tularensis*) that becomes air-borne when mowing lawns where infected/dead animals were present.

Tularemia is frequently diagnosed at the KSVDL in cases received from Southeastern Kansas and neighboring states including Missouri, Arkansas and Oklahoma. A new study conducted at KSVDL has found that there are environmental, climatic, and socio-ecologic conditions that are significant risk factors for this disease in cats. These risk factors include living in newly urbanized areas, or areas surrounded by grassland, or in environments of high humidity. Cats that tested positive for tularemia had experienced significantly higher humidity conditions roughly eight weeks prior to diagnosis compared to those that tested negative.

This study was published recently in the Vector-Borne and Zoonotic Diseases journal by Dr. Raghavan and colleagues and can be found at:

<http://www.ncbi.nlm.nih.gov/pubmed/23593930>.

Future studies are planned to further our understanding of the prevalence of *F. tularensis* among ticks and wildlife in Kansas. The KSVDL is seeking Kansas veterinarians to participate in these studies. If you are interested in participating, please contact Dr. Raghavan at 785-532- 2492 or rkraghavanvet.k-state.edu

KSVDL Anaplasmosis Study

Thanks to all the veterinarians who participated in the KSVDL Anaplasmosis study. We have compiled the information and performed the analysis.

We will be sending a summary of the results to all participating veterinarians in the next couple of weeks.

Thanks Again!!

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Developing, 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.ksu.edu/CE/Conference.htm

Sept. 21 , 2013

SCAAEP Fall Equine Conference

Oct. 9-10, 2013

CEEZAD Mini Symposium

Nov. 9, 2013

KVMA Fall Conference

Nov. 9, 2013

KVTA Fall Conference

Nov. 9, 2013

KHC Equine Clinic: Horse Care 101

Test Results and Schedules

Lab results may be accessed online 24 hours a day, 7 days a week!

To set up an account go to:

www.ksvdl.org

KSVDL will be closed on the following days:

November 21, 22

December 25

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