Tularemia Diagnosed in a Foal and a Cat

We recently identified a case of tularemia in a 1-week-old foal and a cat at Oklahoma Animal Disease Diagnostic Laboratory (OADDL). Tularemia, also known as rabbit fever, is caused by the bacteria *Francisella tularensis*, a highly infectious, zoonotic, gram-negative coccobacillus, that can lead to very severe disease and rapid death in multiple species, including humans. *F. tularensis* is categorized as a tier-1 select agent. Rodents (rabbits, mice, prairie dogs, etc.) and cats are the most susceptible species, but the disease has been occasionally reported in deer, sheep, and dogs, and rarely in cattle and horses.

The foal in this case was presented to the Boren Veterinary Medical Teaching Hospital (BVMTH) for suspected pneumonia and difficulty breathing. Upon presentation, she was systemically ill and had a high fever, despite antibiotic and NSAID treatments. The owner reported that she has removed ticks from the coat. The foal was euthanized three days later due to severe respiratory failure, despite treatments. Gross and microscopic postmortem examination revealed innumerable necrotic foci in lung, liver, spleen, and extensive necrosis in intestinal lymph nodes (Fig. 1), all of which are typical lesions associated with tularemia in susceptible animal species. Similar lesions were observed in the cat submitted to OADDL for necropsy. *F. tularensis* was detected... continued on page 3
Cache Valley Virus, an Emerging Pathogen for Oklahoma?

This article highlights two cases of Cache Valley Virus, identified in two separate flocks of sheep located in Oklahoma, within a month timeframe.

A full-term, 1-day-old, mixed Hampshire/Dorset, male lamb presented for necropsy at the Oklahoma Animal Disease Diagnostic Laboratory (OADDL), out of a total of 7 lambs born with congenital deformities. At the time, infection with Blue Tongue Virus (BTV) was suspected, as symptoms associated with this pathogen had historically been observed in the flock. Gross findings in this lamb included limb malformations (Figure 1), scoliosis (Figure 2), abdominal hernia, hydronephrosis (Figure 3), and hydroureter. Histologic lesions were non-specific, consisting of renal hemosiderosis and fibrosis, hepatic congestion with mild canalicular bile stasis, and residual meconium within pulmonary alveoli. Polymerase Chain Reaction (PCR) detection of BTV was negative; however, Cache Valley viral antigen was positively detected from a fresh sample of kidney.

In the second case, an aborted, Bohr caprine, male fetus presented for necropsy at OADDL. Gross lesions were confined to the presence of peritoneal effusion, and slightly viscous abomasal fluid. Histologically, the most significant findings were confined to the lung with prominent meconium aspiration and vascular congestion. An abortion panel was implemented, in which results were negative for detection of BTV, Bovine Viral Diarrhea Virus (BVDV), Brucella spp., and Campylobacter spp. In the clinical history, congenital limb anomalies were observed in two other newborn goat kids (not submitted for necropsy), and hence, infection with Cache Valley Virus was added to the list of differentials. In a section of fresh brain from this fetus, Cache Valley viral antigen was positively detected via PCR.

Cache Valley Virus is an arbovirus, belonging to the family Bunyaviridae and genus Orthobunyavirus. This virus was first discovered in Cache Valley, Utah, United States in 1956, but since that time, cases have been identified throughout the United States (i.e., Kansas, Texas), Canada, Mexico, the Caribbean and Argentina, being considered endemic within the United States. Viral transmission is commonly via Aedes and non-Culex mosquito vectors, which are typically most prevalent in warm environmental seasons during which mosquito breeding is heightened (i.e., summer to late fall). This virus is particularly devastating when introduced to pregnant ovine or caprine dams during the first trimester of gestation. Experimental evidence indicates that viral infection between 28 and 48 days of gestation, typically results in fetal death, abortion, and/or congenital malformations affecting the musculoskeletal and central nervous systems. Infection after this time interval usually does not result in fetal malformations, and viral antigen levels decline before reaching 70 to 75 days of gestation. Gross lesions include hydro-
Cache Valley Virus, an Emerging Pathogen for Oklahoma?
(continued from page 2)

cerebellus, hydranencephaly, cerebellar and cerebral hypoplasia, arthrogryp-
osis, scoliosis, kyphosis, and skeletal muscle hypoplasia. Histologic lesions include but are not limited to: small diameter skeletal myofibers with loss of striations and infrequent nuclei, thinning of the cerebral cortex with fewer neurons, partial to complete loss of cerebellel lobes, and decreased numbers of axons in the dorsal nerve fiber tracts of the spinal cord.

No vaccines are available to treat Cache Valley Virus infection in sheep and goats once diagnosed, and instead, efforts at disease control and prevention are recommended. Preventative measures include breeding of ewes outside of the mosquito breeding season, and implementing the use of repellants to diminish the mosquito population in affected and endemic areas. Also of utmost concern is the indirect zoonotic potential to humans, via exposure to infected mosquitoes, resulting in disease. Although rare, meningoen-cephalitis and multi-organ failure in humans, have been reported.

— Valerie McElliott, DVM, PhD, Diplomate ACVP

Tularemia Diagnosed in a Foal and a Cat
(continued from page 1)

by PCR and immunohistochemistry (IHC) at OADDL.

_F. tularensis_ is considered a dangerous pathogen as it has a very low infective dose, making it one of the most contagious organisms known to humans. Transmission of the disease can occur by tick or deer fly bites, inhalation of dust or aerosols contaminated with the bacteria, ingestion, skin or eye contact with an infected carcass, or wound inoculation. Ticks of the species _Amblyomma_ spp. (lone star tick) and _Dermacentor_ spp. (dog tick), and deer fly (_Chrysops_ spp.) can acquire and carry the bacteria following ingestion from an infected animal.

Diagnosis of tularemia can be done by culturing _F. tularensis_ or detecting its DNA and antigens by PCR and IHC, respectively, from infected tissues. Because of its zoonotic potential and high infectivity, OADDL performs postmortem examination and handles tissues from suspected animals in the biosafety cabinet and with extra special protection. If you are suspicious of this disease in any animal species, we highly recommend NOT performing a postmortem examination at your clinic and contacting us.

**Take home message:**
Tularemia is a highly infectious, dangerous zoonosis and can lead to severe disease and death in multiple species. Deer flies and certain tick species can transmit the disease to animals and humans, but other routes of infection include inhalation, ingestion, or direct contact when handling an infected animal. Do not open the carcass of a suspected infected animal; contact the diagnostic laboratory to arrange postmortem examination of the animal as a matter of urgency. Tularemia is a reportable disease and positive cases are reported to the State Veterinarian’s office and CDC.

For more information, please visit: [https://www.cdc.gov/tularemia/index.html](https://www.cdc.gov/tularemia/index.html)

— Drs. Giselle Cino, Sai Narayanan, Evan Crisman, Martin Furr, Akhilesh Ramachandran, Girish Patil

New Service Now Available!

To better serve the growing small ruminant industry, OADDL has implemented a small ruminant biosecurity serology panel which includes CAE, caseous lymphadenitis (CL) and Johne’s.
Cytology Corner

The preservation of tissue cells when submitting body fluids for cytologic examination to the diagnostic laboratory need not be a daunting task. It is true; however, tissue cells begin to deteriorate rapidly once outside the body and if they are in a low protein or toxic environment (cerebrospinal fluid, pus, urine) the rate of deterioration happens at an accelerated rate. Here are a few tips that will help get the diagnosis:

1. Always make a direct smear of unconcentrated fluid to send along with the fluid sample. This need only be one air-dried slide.

2. Put the fluid in an EDTA tube (purple top blood tube) immediately after obtaining the sample. The EDTA helps slow cellular deterioration and preserve cell morphology. An added benefit of EDTA is its bacteriostatic property, preventing bacterial overgrowth from occurring while the sample is in transit. Of course, if bacterial culture of the fluid is needed a second culture container would be needed for that submission.

3. Refrigerate (not freeze) your EDTA tube containing the fluid sample until you are ready to send, up to about 4-6 hours. Never put slides in the refrigerator (fluid only) as condensation forming on the slides will lyse unfixed cells.

4. Package the sample with a freezer pack to keep the sample cool in transit.

Alternatively, slide preparation of fluids is relatively easy. Here is how:

1. Make one direct smear of unconcentrated fluid. The unconcentrated preparation allows the clinical pathologist to determine how cellular the sample is and provide an estimated cell count when appropriate.

2. A relatively clear appearance of the fluid means the cellularity is low and a concentrated preparation is needed. The sample can be concentrated the way a urine sediment is made. The concentrated pellet at the bottom of a centrifuged sample is spread on a slide and air-dried.

3. Always note the concentrated preparation on the slide along with patient ID.

4. Include a description of the fluid (e.g. cloudy, clear, white opaque, blood-tinted, etc.) on the accession form.

— Theresa E. Rizzi, DVM, Diplomate ACVP (Clinical Pathology)

What’s your diagnosis? Pleural fluid from a 13-year-old cat.
Find the answer on the last page of this newsletter.
Message from the Director

Dear valued clients and stakeholders:

It is a pleasure to share some information with you once again through this summer 2021 edition of the OADDL newsletter. In addition to the rich scientific content, I wish to share some additional OADDL highlights:

- The Parasitology Laboratory previously managed by the Department of Veterinary Pathobiology became an integral part of OADDL on July 1, 2021. Clients will not notice any changes in services or invoicing. However, this integration will help streamline administrative processes and ultimately serve you better.

- To better serve the growing small ruminant industry in Oklahoma and beyond, we have started offering a small ruminant serology panel, which includes CAE, Johne’s, and caseous lymphadenitis.

- In recognition of the economic disruptions caused by the COVID-19 pandemic, we decided to leave our test fees largely unchanged through June 2022. In the case of Coggins, our fee actually decreased from $12 to $10. This will make it three years since we last raised many of our test fees.

- OADDL discontinued faxing test reports on July 1st to increase reliability of report distribution. All reports will be emailed or mailed ($3 fee, except for regulatory papers). If you are a veterinarian and/or clinic and would like access to your reports and detailed invoices 24/7, please email oaddl@okstate.edu and request access to our Portal.

I want to thank you for trusting us to provide your diagnostic services. I wish you a happy and safe summer. Enjoy the newsletter.

– Dr. Jerry Saliki

Getting to Know Us

Alejandra Medellin is from Tahlequah, Oklahoma. She graduated with a degree in Biochemistry from Oklahoma State University in spring 2019. In her free time, she enjoys being outdoors, running as a hobby and spending time with her family.

Brianna Hamilton joined the Serology Department at OADDL in June 2020 as a Senior Lab Technologist. She received a B.S in Biology and a B.S in Microbiology/Cell & Molecular Biology from Oklahoma State University in 2019.

Brianna’s passions include the ocean, conservation, microbiology and her cat, Poi. Someday she would like to move to the coast and pursue a career in marine conservation/marine biology.

Ideas/Suggestions for Future Content

We want to hear from you. Send your ideas and suggestions to oaddl@okstate.edu.

Contact Us

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Cytology Corner Answer: Chyloous effusion—chylomicron rich fluid with triglyceride concentrations higher than plasma concentration. This effusion is typically lymphocyte-rich, but with a long-standing or recurrent effusion, neutrophils will increase. In cats, it is often associated with heart disease. Fun fact—the lipid interferes with refractometer readings of total protein falsely elevating the value!