Tularemia kills group of cats

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Recently we received tissues from a cat that was one of a group of six young adult cats to die within 5 days. Clinical symptoms included lethargy, anorexia, labored breathing, and nasal and ocular discharges. At necropsy the attending veterinarian noted that the mesenteric lymph nodes and liver were enlarged and the lungs were edematous. While processing the tissues at the lab, it was also noted that there were hemorrhages in the mesenteric lymph nodes and small intestine, and that the spleen contained many white spots.

There are not a lot of things that kill this many adult cats this quickly. One cause is always some type of toxin, but as far as infectious diseases, in this region there is feline panleukopenia and tularemia and not much else. Plague, caused by *Yersinia pestis*, is another possibility and it can be found in the very western parts of Kansas, but in the past 13 years we have not had a case that I know about. The cats had been vaccinated twice for panleukopenia, which made that unlikely.

The white spots in the spleen and hemorrhagic, enlarged lymph nodes were suggestive of tularemia, and *Francisella tularensis*, the cause of tularemia, was isolated from the cat’s tissues. When tularemia was first suspected the veterinarian was contacted in an effort to warn him. The veterinarian said that he was treating another cat from the same household with gentamicin and intravenous fluids, and the cat was beginning to recover.

Aminoglycoside and fluoroquinolone antibiotics are considered to be the antibiotics of choice. Tetracyclines are also used successfully, but the incidence of relapses is higher. Penicillins and cephalosporins are ineffective.

It is not uncommon for us to receive tissues from cases where multiple cats have died of tularemia in just a few days. In these cases, infection is probably by ingestion of a rabbit or some other animal infected with tularemia. Tick bites are the other common method of transmission.

Fifty years ago the most common method for people to acquire tularemia, or rabbit fever as it was commonly called, was skinning and cleaning rabbits. Today with much less rabbit hunting, the most common method is tick bite.

Cats and rabbits are not the only animals that can transmit tularemia. Recently there have been news reports of sheep dying in Wyoming of tularemia. I know a pathologist at another university who acquired tularemia when he cut himself while collecting samples from a deer to test for chronic wasting disease. The wound did not heal, and in a couple of days he was feverish and the draining lymph nodes were swollen and painful. Tularemia is a serious disease and if anyone suspects it they should see a physician immediately.

However, there are multiple reports in the literature of cat to human transmission by cat bite, so infected cats should be handled carefully. The veterinarian said that he was treating another cat from the same household with gentamicin and intravenous fluids, and the cat was beginning to recover.

Adding Value to Calves is the general session topic for a new two-day Animal Sciences and Industry conference to take place August 9-10 in Manhattan. This conference is designed to provide information to help cow/calf producers improve profitability.

On Thursday industry experts will present information on the current beef situation and calf market outlook followed by information on practical methods to add value to calves in a declining market. Dr. Bill Mies will be the keynote speaker.

Concurrent demonstrations Friday morning include cattle handling, live animal evaluation and carcass end products, and practical cow feeding including ration formulation exercises.

Conference information can be found at [http://www.asi.ksu.edu/](http://www.asi.ksu.edu/) under Upcoming Events. For registration information contact Linda Siebold at 785-532-1281 or lsiebold@oznet.ksu.edu.
Diagnosis, treatment of bovine anaplasmosis in beef herds

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Anaplasmosis, caused by the rickettsial hemoparasite *Anaplasma marginale*, is the most prevalent tick-transmitted disease of cattle worldwide and a major obstacle to profitable beef production in the continental United States (Uilenberg, 1995; Dumler et al., 2001; Kocan et al., 2003). Anaplasmosis is readily transmitted through biological and mechanical vectors such as ticks and biting flies and iatrogenically through needles and equipment contaminated with infected blood.

Clinical anaplasmosis, characterized by anemia, icterus and fever, is associated with significant production losses, abortions and mortalities in cattle. It is estimated that the introduction of anaplasmosis into a previously naïve herd can result in a 3.6% reduction in calf crop, a 30% increase in cull rate and a 30% mortality rate in clinically infected adult cattle (Alderink et al., 1982). Furthermore, a study has shown that 16% of pregnant carrier cows will transmit anaplasmosis in utero producing persistently infected offspring (Potgieter et al., 1987). The existence of both horizontal and vertical anaplasmosis transmission has important implications for disease control in endemic areas. The cost of a clinical case of anaplasmosis in the USA has been conservatively estimated to be more than $400 per animal with some estimating the total cost to the beef industry to be more than $300 million per year (Goedger et al., 1979; Alderink et al., 1982; Kocan et al., 2003).

In addition to the costs associated with clinical anaplasmosis, animals recovering from acute anaplasmosis, including those treated with recommended doses of tetracyclines, remain lifelong *A. marginale* carriers. There are currently no antimicrobial compounds approved for elimination of persistent *A. marginale* infections in cattle, despite published reports of successful carrier clearance with tetracyclines. Carrier animals serve as reservoirs of infection for mechanical transmission and infection of ticks (Eriks et al., 1989; Palmer et al., 2000; Kocan et al., 2003). This restricts the export of cattle from endemic areas such as the United States to non-endemic territories such as Canada. Anaplasmosis is therefore a significant impediment to unrestricted international movement of cattle in North America. Successful measures to control and eradicate anaplasmosis are confounded by the absence of efficacious antimicrobial regimens to eliminate infections (Coetzee et al., 2005), inadequate information regarding the usefulness of newer diagnostic tests in determining the success of disease eradication, and ineffective vaccines to protect against new infections.

**Diagnosis of Anaplasmosis**

Members of our research group conducted a study to compare the sensitivity of the complement fixation (CF) and a new competitive enzyme-linked immunosorbent assay (cELISA) tests for detection of *A. marginale* in experimentally infected steers (Coetzee et al, 2007). Forty Angus X Simmental steers were experimentally infected with 2.6 x 10^9 A. marginale infected erythrocytes. Percent parasitized erythrocytes (PPE) were determined by microscopic examination and sera were tested by CF and cELISA using USDA-approved methods from blood collected at 9, 13, 20, 28, 34, 41, 61, 96, 126 and 156 days post infection (DPI). At 9 DPI, sensitivity of the cELISA test was 47.5% whereas the CF test failed to identify positive animals. After 13 DPI, sensitivity of the cELISA and CF test were 100% and 20%, respectively, during peak parasitemia (20 DPI), each test had a sensitivity of 100%. Thereafter, sensitivity of the CF test fluctuated between 7.5% and 37.5% while the cELISA test remained at 100%. The overall sensitivity of the cELISA and CF tests was 94.8% and 26.5%, respectively with a kappa statistic of 0.039.

These results indicate that the cELISA has superior sensitivity for the serological detection of *A. marginale*. It is however significant that both tests demonstrated a high percentage of false negatives during the prepatent period. For the purpose of identifying anaplasmosis carrier cattle, this new commercially available cELISA test now offered by the Veterinary Diagnostic Laboratory at Kansas State University is reported to have a sensitivity of 96% and specificity of 95%.

Microscopic examination of stained blood films is commonly used to detect *A. marginale* organisms in erythrocytes of infected animals. However, this diagnostic technique may be unreliable when cattle have minimal infections or in advanced cases of the disease when animals are severely anemic.

In the study described previously, we observed that the cELISA accurately identified all infected cattle before the number of *A. marginale*-infected erythrocytes exceeded a PPE of 1%. This suggests that the cELISA may be more sensitive than examination of stained blood films for identifying early clinical cases.

Furthermore, in instances in which the PPE is low, intraerythrocytic inclusions of *A. marginale* may easily be confused with Howell-Jolly bodies, basophilic stippling of reticulocytes, and stain contamination. This suggests that the cELISA may be a useful alternative to examination of stained blood films for the diagnosis of anaplasmosis, especially in situations in which experience of clinicians or the available facilities are inadequate for interpretation of blood films.

Having said this, veterinarians should exercise caution before making a definitive diagnosis of acute anaplasmosis solely on the basis of a positive result for the cELISA and the presence of clinical signs such as fever, anemia, and icterus. *A. marginale* carrier cattle are cELISA posi-
tive but not rickettsemic and therefore do not develop anemia and icterus associated with erythrophagocytosis of parasitized erythrocytes. Differential diagnoses that should be ruled out based on these clinical signs include acute anthrax, leptospirosis, bacillary hemoglobinuria, oak poisoning, poisoning caused by ingestion of Brassica species and multicentric lymphosarcoma. In these circumstances, it would be advisable to detect intraerythrocytic inclusions of A. marginale by examination of blood films to assist in differentiating between anaplasmosis and these other diseases.

Molecular biological tests appear to be the future of definitive anaplasmosis identification and control strategies in very early stages of infection. Currently, polymerase chain reaction (PCR) is an area that is receiving the attention and focus of research efforts at K-State (Sirigireddy and Ganta, 2005). PCR utilizes biochemical and molecular biological processes to amplify the genetic material of an organism. DNA-based PCR for identification of A. marginale is presently being used based on previous publications (Torioni De Echaide et al. 1998).

Research efforts at K-State are focused on developing a highly sensitive and specific diplex, RNA-based PCR diagnostic tool for identification of both A. marginale and A. phagocytophilum infections. The enhanced sensitivity of RNA-based versus DNA-based PCR is derived from the typical ratio of RNA: DNA molecules per organism being on the magnitude of 100:1. Torioni De Echaide (1998) and others report a sensitivity of 30 infected erythrocytes per milliliter of blood for the DNA-based PCR. This translates to 30 molecules of DNA and 3,000 of RNA.

Preliminary results for the RNA-based PCR test are projected to detect an infection with even fewer infected erythrocytes per milliliter of blood. Also, the RNA target within each respective organism is highly conserved and specific among isolates and provides for accurate and precise identification of infective organisms. RNA-based test results will provide a positive or negative diagnosis as well as an estimate of the number of infective organisms in the sample. The currently available DNA-based test result only yields a positive or negative test result.

### Treatment of Persistent Anaplasmosis

Chlortetracycline (CTC) and oxytetracycline (OTC) are the only compounds approved for use against acute anaplasmosis in the United States. In regard to the oral administration of oxytetracycline or chlortetracycline, there are currently no compounds approved for the elimination of the carrier state in the USA (Bayley, 2005). Current label claims for chlortetracycline (Aureomycin 90, Alpharma) are as follows:

**Beef Cattle (over 700 lb):**
Control of active infection of anaplasmosis caused by Anaplasma marginale susceptible to chlortetracycline – 0.5 mg/lb Chlortetracycline body wt/day.

**Beef and Non-Lactating Dairy Cattle (over 700 lb):**
Control of active infection of anaplasmosis caused by Anaplasma marginale susceptible to chlortetracycline when delivered in a free-choice feed. Free-choice feed must be manufactured under a feed mill license utilizing an FDA approved formulation – 0.5 to 2.0 mg/lb Chlortetracycline body wt/day.

Published studies that claim to have achieved successful clearance of carrier infections used the following variations of labeled dose regimens (Kuttler, 1980):
- Chlortetracycline 2.2 mg/kg (1 mg/lb) orally daily for 41 days;
- Chlortetracycline 1.1 mg/kg (0.5 mg/lb) orally for 120 days.

It is evident from this review that there is a paucity of scientific evidence to support the use of presently approved CTC dose regimens for clearance of persistent anaplasmosis infections. Our research group is currently studying these clearance strategies under experimental conditions in order to develop more scientifically validated therapeutic regimens.

At this time, if carrier clearance is to be attempted, we suggest that blood samples are collected from the herd for cELISA testing before CTC treatment commences. This will allow producers to determine the seroprevalence of anaplasmosis in the herd and to judge the success of therapy. We believe that feeding 2.2 mg/kg CTC for at least 4 months during the non-vector season would offer the best chance of a favorable therapeutic outcome. If active anaplasmosis infections are present in the herd, especially if there are a number of animals in the prepatent, patent or early recovery phase, the efficacy of CTC treatment will be significantly reduced.

Tetracycline treatment in the prepatent period has been shown to simply delay the onset of clinical disease. It should also be remembered that up to 16% of calves born to carrier cows could be persistently infected without showing any signs of the disease. Preliminary studies suggest that a follow-up blood sample tested with the cELISA test at 3 to 6 months after treatment will provide serological evidence of whether carrier clearance with CTC was successful. Producers in endemic areas should be advised that such clearance strategies could result in an unstable situation arising with respect to herd immunity which may give rise to disease outbreaks should anaplasmosis be reintroduced in the herd.

Once the RNA-based PCR test is fully developed, our research group will examine the performance of these assays on calves experimentally-infected with A. marginale that are being fed chlortetracycline at different dosage levels. Our goal is to be able to provide veterinarians and livestock producers in Kansas with science-based recommendations for controlling and perhaps eliminating persistent anaplasmosis infections from their herds.

These tests will not only validate the diagnostic power of the RNA-based PCR test, but will provide a useful tool for quantifying the number of A. marginale organisms per volume of blood as well as track changes in the number of A. marginale organisms throughout treatment with oral chlortetracycline. The data produced from these efforts will provide valuable and definitive scientific information regarding carrier clearance and the true effect of treatment with chlortetracycline on cattle infected with A. marginale.

### References


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Bracken fern toxicosis in Kansas cattle

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A yearling beef bull was presented to the Kansas State University Veterinary Teaching Hospital for evaluation of epistaxis, fever and depression. A pasture mate had died acutely two days prior with similar symptoms plus frank blood coming from the rectum. One year previously, during the same time of year, a similar clinical picture was seen in another bull, but the cause was undetermined. The on-farm post-mortem exam revealed significant hemorrhage within the abomasum and large bowel. The problems were confined to a group of young bulls pastured and fed together with commercial supplements and home-grown hay.

The bull presented to the hospital had an elevated heart rate (95 beats per minute), respiratory rate (50 breaths per minute), temperature (104°F), and moderate bilateral epistaxis. The bull had been treated with antimicrobials and anti-inflammatory drugs before admission.

A mild decrease in packed cell volume (24%, normal 26-42), normal total plasma protein (7.3g/dl), and a prolonged clotting time were determined. The initial clotting time was performed using a modified Lee-White method in which blood collected into a red top tube normally clots in less than 5 minutes. The patient’s blood failed to clot within 20 minutes. Blood loss anemia was suspected and the most likely differential diagnoses were (type II) BVD induced thrombocytopenia, moldy sweet clover (dicoumerol) poisoning, abomasal ulceration, and liver abscesses. Less likely differential diagnoses including myelodysplasia, immune mediated disease, and rare toxins.

The cause of the prolonged clotting time was investigated. Intrinsic and extrinsic clotting times were consistent with healthy control animals, thus eliminating factor deficiency diseases such as that caused by moldy sweet clover. A CBC revealed marked thrombocytopenia, mild normocytic normochromic anemia, and a white blood cell differential containing only atypical reactive lymphocytes. There were zero neutrophils, monocytes, cosinophils, or basophils identified in the sample.

The erythrocyte morphology indicated marked poikilocytosis and crenation. The CBC results indicated decreased erythropoiesis and granulopoiesis. The presence of only lymphocytes was likely due to the longer peripheral lifespan compared to the granulocytes. The peripheral lifespan of lymphocytes can be weeks to months compared to the short transit times and unidirectional tissue migration of other white blood cells.

The CBC findings elevated a bone marrow suppression disease such as myelodysplasia as a differential. Further questioning about the farm environment indicated that groves of bracken fern were present around the barn and periphery of the pasture where these bulls were kept. Also, these cattle were fed hay harvested from a pasture immediately adjacent to where the bulls were kept.

Within 36 hours of admission the bull had a fever of 105°F, and the clinical picture indicated a grave prognosis. The bull was humanely euthanized and necropsied in search of a definitive diagnosis and recommendations to be made in regarding the remainder of the herd in question. The most likely differential diagnosis was a disease causing bone marrow suppression.

Two of the more likely causes of bone marrow suppression in cattle are bracken fern toxicosis and trichloroethylene toxicosis. Decades ago trichloroethylene was commonly used in the extraction process of cattle feeds such as soybean meal. Cattle fed feed processed in this manner developed renal damage and fatal aplastic anemia. Trichloroethylene exerts its effects more profoundly on the erythroid precursors than granulocytic precursors and also causes significant renal damage (Lock et al., 1996). Trichloroethylene however may be present as an environmental contaminant (Ou et al., 2003).

Bracken fern has two hemolymphatic syndromes associated with it, acute toxicity as well as enzootic hematuria. Both result from chronic consumption of either live or dead plant material. Cattle consuming nearly their body weight in bracken fern over 3 weeks to 3 months can develop acute clinical bracken poisoning. Despite being categorized as an acute syndrome, the clinical signs of the disease may appear only several weeks after the consumption of the plant. Lightweight cattle developing a taste for the bracken fern are usually affected by the syndrome. Cattle consuming 1-3% of their body weight over months to years can develop enzootic hematuria which begins has a hemorrhagic cystitis (Lock et al., 1996; Ou J et al, 2003). Other syndromes include retinal degeneration in sheep, GI neoplasia, and signs of polioencephalomalacia in horses due to the thiaminases in some species of fern. Enzootic hematuria is seen as frank blood in the urine, stranguria and is also associated with urinary bladder neoplasia, most commonly with concomitant infection by bovine papilloma virus-2.2. Bracken poisoning is associated with bone marrow suppression affecting granulocyte precursors more rapidly and severely than erythrocyte precursors. Lack of circulating granulocytes makes the animal more susceptible to secondary infection leading to the clinical signs of fever and depression. Thrombocytopenia leads to systemic hemorrhage. The toxic compound found in the fern and utilized to reproduce disease experimentally is ptaquiloside.

Post-mortem diagnoses were panhypoplasia of the bone marrow, disseminated ecchymotic and petechial hemorrhages, ulcerative colitis with bacterial invasion, and necrotizing lymphadenitis with bacterial colonies. The bone marrow was compared to that of two other bovines and there was a decrease in cellularity of granulocytic and erythrocytic cell lines, with more marked decrease in granulocytic cells. Granulocytes in the bone marrow were negligible, whereas normally granulocytes should make up 40% of nucleated cells. Fluorescent antibody tests on tissues for BVD were negative.

The diagnosis of acute bracken poisoning was thus made based on clinical signs, ante-mortem diagnostics, plant exposure and post-mortem findings. Specific ante-mortem tests for this condition, such as measuring ptaquiloside, are unavailable. Unsuccessful attempts were made to identify bracken fern within the rumen.

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contents. Prolonged exposure and diluted samples by the time disease was evident makes finding the plant in the rumen difficult. In the United States, the condition is most commonly associated with animals in the Pacific Northwest, where climate permits growth of large stands of the plant. However, moist, low-lying areas and open woodlands, which are the preferred growth conditions, can be found in most of the country. Dried plant material in hay retains the ability to cause disease as well. There is no specific therapy other than supportive care once clinical disease is apparent. Recommendations are limited to preventing further exposure and client education about clinical syndromes and risk factors.

References

Recommended Reading

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Goat abortions caused by caprine herpesvirus

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Recently, we diagnosed caprine herpesvirus in several goat fetuses that were from a flock in which more than 25% of the pregnant females had already aborted. Last winter we diagnosed caprine herpesvirus in goat fetuses from a flock where the owner said that they had a wheelbarrow full of aborted and stillborn kids. Caprine herpesvirus is an alpha herpesvirus that is closely related to and partially cross-reacts with infectious bovine rhinotracheitis (IBR) virus. Like other alpha herpesviruses such as IBR virus, equine herpesvirus, and pseudorabies virus, caprine herpesvirus can cross the placenta, infect fetuses, and cause large abortion storms in groups of naive animals. Also similar to other alpha herpesviruses, caprine herpesvirus causes viremia and death in neonates. At necropsy, gross lesions in viremic neonatal kids often consist of ulcers and erosions throughout the gastrointestinal tract.

In adults, caprine herpesvirus can cause erosions and ulcers of the prepuce, penis, vulva, and vagina, or infection can be asymptomatic. In many cases abortion is the only clinical manifestation. Like all herpesviruses, after the initial infection caprine herpesvirus becomes latent and infection is life long. During times of stress the virus can become reactivated and be transmitted to susceptible animals in the flock. Therefore, once a flock is infected the virus is maintained in the flock as long as there are infected animals.

Published reports of caprine herpesvirus infection are rare but as the number of goats in the United States increases, the incidence of caprine herpesvirus outbreaks will likely increase. If the number of goats seen at the K-State Veterinary Diagnostic Laboratory is representative, the number of goats, especially meat goats, is increasing rapidly.

Goat abortions caused by caprine herpesvirus