Anaplasmosis: Practical principles for the diagnosis, treatment and control of field cases and outbreaks

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Abstract

Anaplasmosis, caused by the rickettsial hemoparasite *Anaplasma marginale* (Am), is the most prevalent tick-transmitted disease of cattle worldwide and a major obstacle to profitable beef production in the continental United States. 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. Furthermore, a study has shown that 16% of pregnant carrier cows will transmit anaplasmosis in utero producing persistently infected offspring. The existence of both horizontal and vertical anaplasmosis transmission has important implications for disease control in endemic areas. In this presentation, we will use case studies to examine strategies to treat and control anaplasmosis in beef and dairy herds.

Key words: anaplasmosis, outbreaks, biosecurity, cELISA

Introduction

Anaplasmosis is one of the most challenging diseases facing cattle producers worldwide.[1,8] After infection, there is typically a 4- to 8-week incubation period before clinical signs are observed (Figure 1).[7] During this time, cattle often test negative for Am on diagnostic tests. This may lead to the introduction of recently exposed cattle into a naïve herd in spite of pre-movement serological testing. Clinical anaplasmosis causes production losses, abortions and mortality in cattle.[1,8] Cattle that recover from acute anaplasmosis maintain a microscopically undetectable parasitemia for life. Persistent infection is characterized by sequential rickettsemic cycles ranging from 102 to 107 that occur at approximately 5-week intervals.[6] Carrier infections confer resistance to clinical anaplasmosis leading to endemic disease stability; however, deaths may still occur during times of stress or following introduction of naïve animals to an infected herd. Am infections may be transmitted mechanically, through biting flies or equipment contaminated with infected blood; biologically via ticks or transplacentally to unborn calves. Ticks that become infected after feeding on carrier cattle may attach to wildlife including deer and spread anaplasmosis across fence lines to neighboring livestock. Successful measures to control and eradicate anaplasmosis are confounded by vaccines that are ineffective because they fail to protect against new infections and the absence of validated antimicrobial regimens to eliminate existing infections.

Chlortetracycline (CTC) and oxytetracycline (OTC) are the only compounds approved to control anaplasmosis in the U.S. Therefore, it is critical that their efficacy be preserved.[2,8] Enrofloxacin (Baytril CA®, Elanco) was recently approved to treat acute anaplasmosis infections in replacement dairy heifers under 20 months of age, and all classes of beef cattle except beef calves less than 2 months of age and beef bulls intended for breeding (any age).

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.[2] 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. Successful measures to control and eradicate anaplasmosis are confounded by the absence of efficacious antimicrobial regimens to eliminate infections, 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

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

Figure 1: Comparison between complement fixation (CF) and competitve ELISA (cELISA) sensitivities (Se), Mean Percent Parasitized Erythrocytes (PPE) and Packed Cell Volume (PCV) following infection with 2.6 × 109 *A. marginale* infected erythrocytes.

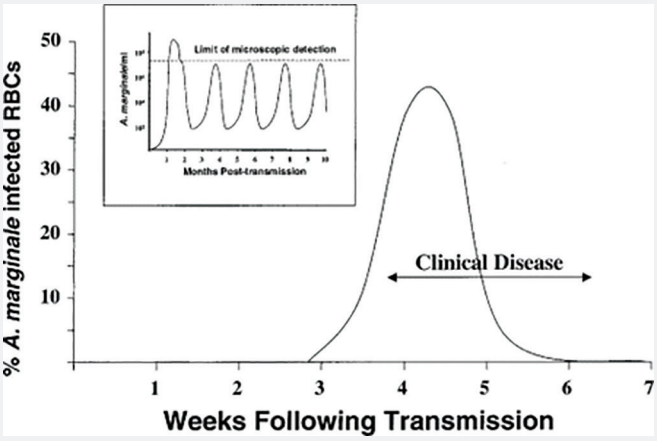

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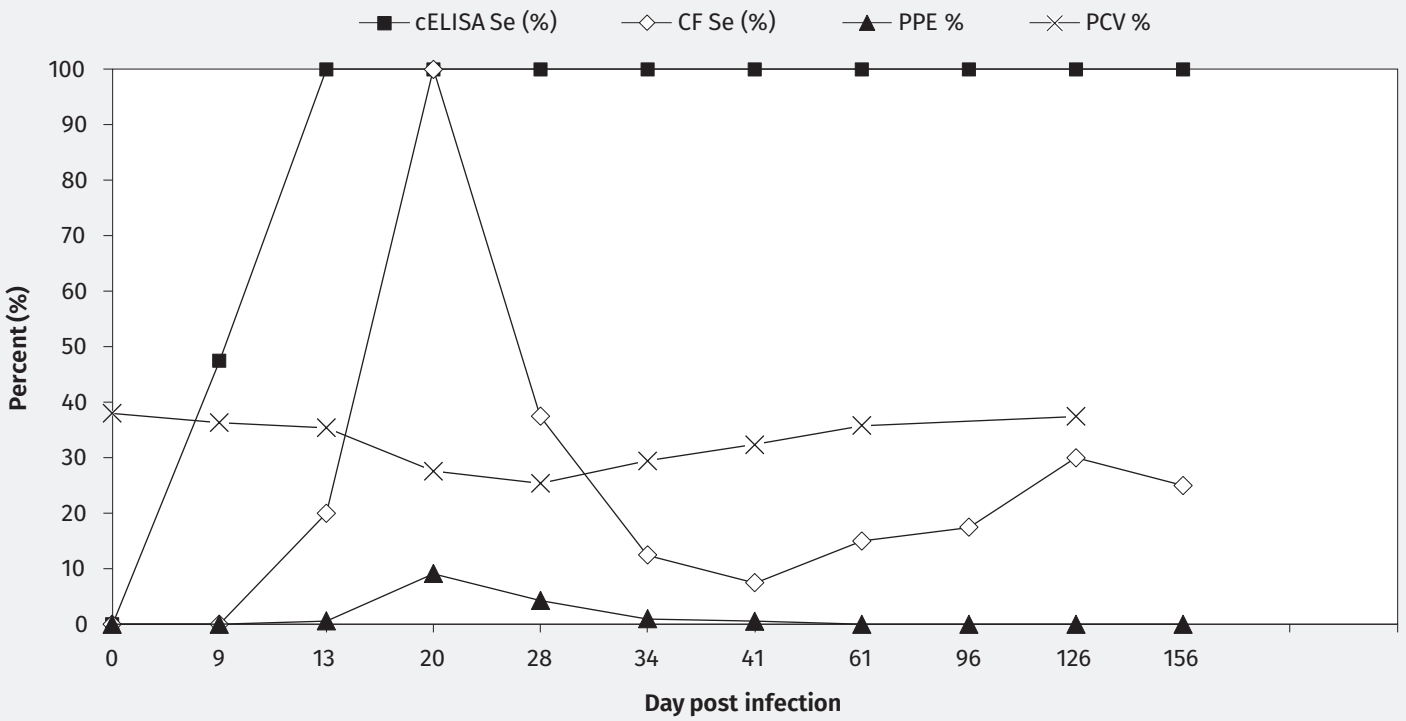
Figure 2: Comparative sensitivities of cELISA and CF tests compared with PPE Post Infection



of *A. marginale* in experimentally infected steers (Figure 2).[2] Forty Angus x Simmental steers were experimentally infected with 2.6 × 109 *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 d 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 from 7.5% to 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 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 situation in which experience of clinicians or the available facilities are inadequate for interpretation of blood films.

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 Kansas State. 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. Present research efforts at Kansas 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.[13] This translates to 30 molecules of DNA and 3,000 molecules 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.

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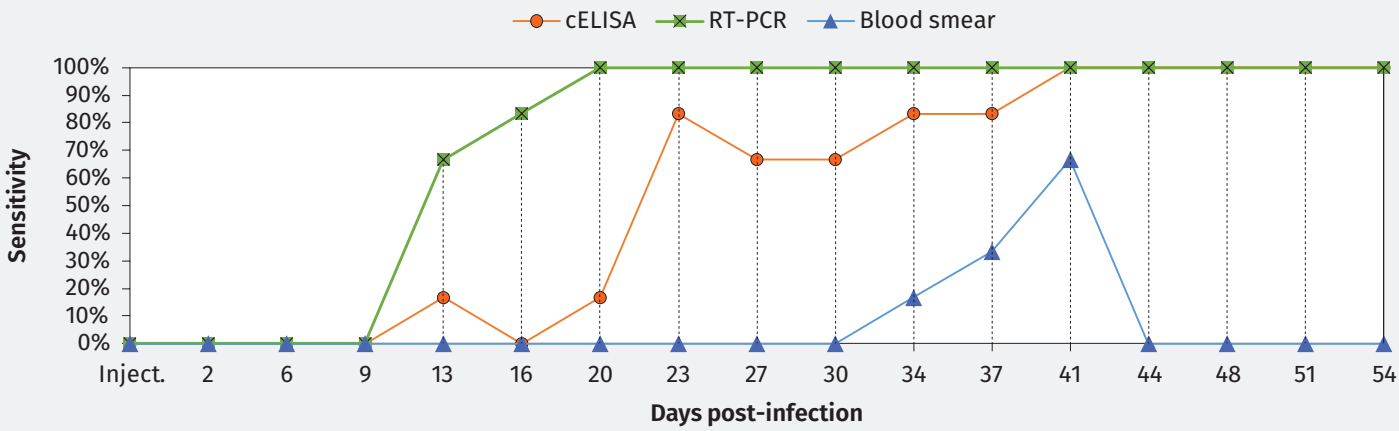
Figure 3: Comparative diagnostic sensitivity of the RT-PCR assay, competitive ELISA (cELISA) assay and modified Wright's stained blood smears following a single injection with an A. marginale contaminated 16G, 1" needle in Group B.



Significance of iatrogenic anaplasmosis transmission

The significance of iatrogenic transmission has recently been demonstrated in two studies.[9,10] This study compared iatrogenic transmission of *A. marginale* during simulated vaccination between needle and needle-free injection techniques and diagnostic method performance of light microscopy, cELISA and an *A. marginale*-specific RT-PCR assay (Figure 3). Twenty-six Holstein steers confirmed negative for anaplasmosis by cELISA and RT-PCR were infected with a Virginia isolate of *A. marginale* propagated to a circulating parasitemia of 2.0% in a splenectomized steer (SPS). A simulated vaccination of the infected steer was conducted by IM injection using a hypodermic needle fitted to a multi-dose syringe. The same needle and syringe were utilized to sham "vaccinate" a naïve steer. This two-step procedure was repeated until 10 naïve steers (ND) were injected. Similarly, the right neck muscles of the SPS were injected by a needle-free injection system for a separate group of 10 naïve calves (NF). Five calves remained non-injected, sentinel steers (CONT). Disinfectants were not used during the procedure. Disease status was monitored semi-weekly during a 61-d study by light microscopy, cELISA and RT-PCR. Iatrogenic transmission occurred in 60% of steers in the ND group. No change in disease status occurred in the NF or CONT groups. Light microscopy, cELISA and RT-PCR demonstrated 100% sensitivity on Day 41, 41 and 20 post-infection, respectively; however, only cELISA and RT-PCR sustained 100% sensitivity thereafter. Needle-free injection was shown to be superior to needle injection for controlling iatrogenic transmission of *A. marginale*. The sensitivity of cELISA and RT-PCR were similar following the acute phase of infection.

Impact of bovine anaplasmosis on dairy herds

We recently published a survey to investigate the within-herd seroprevalence of antibodies to Am and the relationship between disease status and milk production after anaplasmosis outbreak in a northern Iowa dairy herd.[3] In 2010 anaplasmosis was diagnosed in an Iowa dairy herd composed of 680 lactating Holstein cows. Samples for serological testing by competitive ELISA were gathered from 799 animals throughout 2011 in 24 separate accessions. Information on milk production, obtained from the DHIA, was gathered from 2010 to 2013. Monthly DHIA milk production was then statistically compared with 2011 anaplasmosis serostatus. Analysis of competitive ELISA data found that 38% of the animals tested positive for bovine anaplasmosis. The DHIA milk data showed seropositive cows produced significantly less milk during 2012 ($P = 0.0041$) and 2013 ($P = 0.0351$) than did seronegative animals. This resulted in a mean (±SEM) difference of 1,677 ± 579 kg and 2,175 ± 1,022 kg of milk per cow during 2012 and 2013, respectively. Cows found to be seropositive for Am antibodies produced significantly less milk in subsequent lactations than seronegative cows. Therefore, subclinical anaplasmosis may represent a potential loss of income for dairy producers. Results also suggest that animals should not be assumed free of infection based on geographic location.

Impact of bovine anaplasmosis in beef herds

Recently we assessed within-herd seroprevalence of *A. marginale* antibodies across 12 Florida beef cattle herds and compared this with statewide seroprevalence.[5] Twelve surveyed herds ranged in size from 160 to 456 adult *Bos taurus-Bos indicus* cattle. Screening relied on competitive ELISA. Before serology, an outbreak of anaplasmosis resulted in increased mortality (up to 17.8%) and abortions in several herds. Up to 29.2% of cows aborted late in gestation in two herds that included many cattle introduced from Texas. Among 1,085 cattle tested in the 12 herds, seroprevalence of *A. marginale* varied from 2.6 to 85%, with an overall seropositive rate of 50.3%. Cattle in open herds were 6.23 (95% CI: 4.26–9.17) times more likely to experience mortality and 3.10 (95% CI: 2.39–3.98) times more likely to abort than animals in closed herds. Average mortality (12%) and abortion (16.3%) among open herds were significantly ($P < 0.05$) higher than mortality (1.9%) and abortion (5.3%) among closed herds. These data highlight unrestricted cattle movement and environmental conditions that favor vector-borne disease transmission as risk factors for disease outbreaks even in regions that are considered endemic for bovine anaplasmosis.

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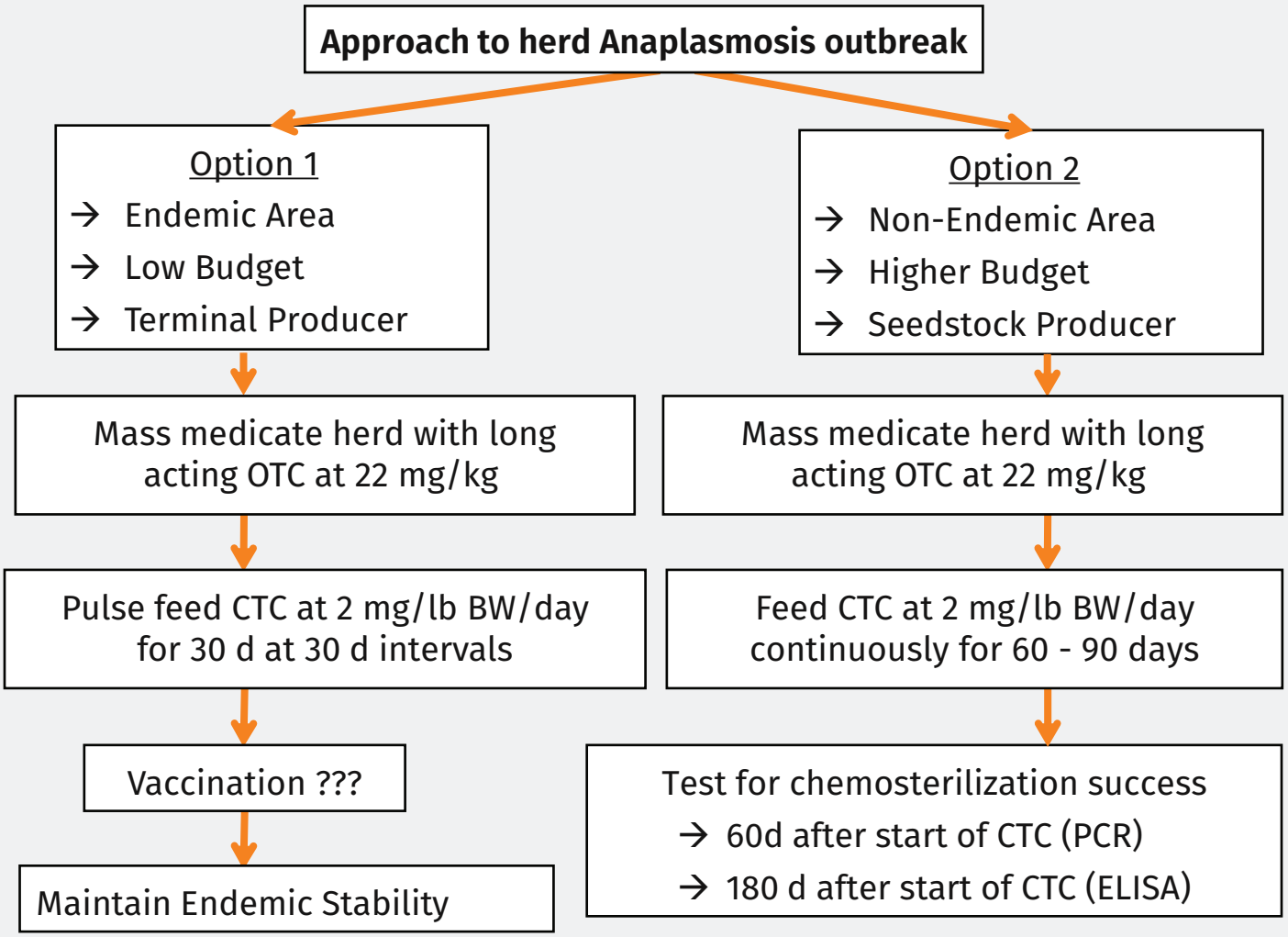
Figure 4: Approach to Herd Anaplasmosis Outbreak



Treatment of persistent anaplasmosis infections

Chlortetracycline (CTC), oxytetracycline (OTC) and enrofloxacin (Baytril CA, Elanco)[12] 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 U.S. 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./d.

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/d."

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

Chemosterilization has been reported in cattle fed chlortetracycline hydrochloride (CTC) at dosages ranging from 1.1 mg/kg for 120 d to 11 mg/kg for 30 to 60 d. The relationship between plasma CTC drug concentration and carrier clearance has not been described until recently.[11] In a study conducted by our research group, chronic carrier status was established in 21 steers with a Virginia isolate of *A. marginale* and confirmed by cELISA and the previously described *A. marginale* -specific RT-PCR.[12] Four naïve, splenectomized steers served as active disease transmission sentinels. Steers were randomized to receive either 4.4 mg/kg/d (LD); 11 mg/kg/d (MD); or 22 mg/kg/d (HD) of oral chlortetracycline; or placebo (CONTROL) for 80 d. The LD, MD and HD treatment groups consisted of five infected steers and one splenectomized steer; CONTROL group had 6 infected steers and 1 splenectomized steer. The daily treatments and ration were divided equally and fed twice daily. Blood samples were collected semi-weekly for determining plasma drug concentration by ultrahigh performance liquid chromatography-mass spectrometry/mass spectrometry method and assessment of disease status by both cELISA and RT-PCR. Mean (CV%) chlortetracycline plasma drug concentrations in the LD, MD and HD groups were 85.3 (28%), 214.5 (32%) and 518.9 (40%) ng/mL from Day 4 to Day 53 of treatment. A negative RT-PCR assay result was confirmed in all CTC-treated groups within 49 d of

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treatment; however, cELISA required an additional 49 d to 88 d before similar results. Subinoculation of splenectomized steers confirmed chemosterilization. These results demonstrate that CTC may be used to eliminate persistent *A. marginale* infections but cattle are susceptible to reinfection with anaplasmosis after clearance. These data are important for influencing future chemosterilization strategies and impacting free trade policy among countries and regions of contrasting endemicity.

It is noteworthy that the manner in which chlortetracycline was administered in this study is not consistent with how CTC is administered in accordance with the requirement of most veterinary feed directives. Therefore, we recently conducted a study to determine the effect of approved oxytetracycline and chlortetracycline indications on *A. marginale* bacteremia.[4] Fifteen animals with persistent anaplasmosis were enrolled and divided into 3 treatment groups. Group 1 (n = 6) received oral chlortetracycline (1.1 mg/kg bodyweight) administered via hand-fed medicated feed for 60 d. Group 2 (n = 6) received injectable oxytetracycline administered subcutaneously at 19.8 mg/kg bodyweight 3 times in 3-week intervals. Group 3 (n = 3) served as an untreated control. After 60 d, bacteremia failed to permanently decrease in response to treatment. This result indicates that clearance of *A. marginale* is unlikely to be reliably achieved using currently approved tetracycline-based regimens to manage anaplasmosis.

The approach to an outbreak of anaplasmosis is presented in Figure 4. Option 1 is recommended in an endemic area, where the producer is on a low budget or they're a terminal producer. If there are a large number of cattle dying from anaplasmosis, I would recommend mass-medicating with long-acting tetracycline and then pulse feeding CTC at 2 mg/kg bodyweight/d for 30 d taking a 30-d break and then pulse feeding again for 30 d throughout the vector season. I would not recommend continuous feeding of CTC because I am concerned that we could be inadvertently chemosterilizing those cattle making them completely susceptible to reinfection with anaplasmosis in subsequent seasons. In an endemic area, that's the last thing you want to do is create endemic instability. The other option is vaccination. There are currently no USDA approved vaccines to prevent anaplasmosis in cattle and so you will have to obtain conditional USDA approval to use the vaccines that are available, especially in certain states. Current vaccines may prevent animals from dying, but these do not prevent cattle from becoming carriers.

If you are a seed stock producer, you probably wouldn't want to use the vaccine because animals may become seropositive, but if you are in an endemic area, you may want to consider the vaccine however, this only contains one isolate of anaplasmosis and may not cross-protect between all strains. I don't currently recommend vaccination, but it is something you can consider if other control measures are unsuccessful. Pulse feeding is what I would recommend to maintain that endemic stability, but also to control the organism sufficiently to prevent those animals from dying. If you're in a non-endemic area, with a higher budget, and a seedstock producer, you could consider mass medicating with injectable long acting OTC at 22 mg/kg and then feeding CTC at 2 mg/lbwt/d continuously for 60 to 90 d (Option 2). Then you can test to see if you were successful for chemosterilization 60 d after start of CTC with PCR or 120 d after the start with the cELISA test.

There are several potential reasons why chemosterilization can be unsuccessful. The most common cause of unsuccessful chemosterilization is inadequate drug intakes when these are administered orally. Personally, I don't believe a medicated mineral constitutes an adequate means of delivering CTC for anaplasmosis control in many cases. Chemosterilized cows could also become reinfected after treatment and that could result in treatment failures. We continue to investigate if resistance to tetracyclines could be another potential cause of treatment failure.

Comparison of various anaplasmosis control strategies

Below is a table I compiled for presentation at the Kansas State Anaplasmosis D IN 2019 with projected estimates of the cost of 3 anaplasmosis control strategies for a hypothetical herd of 100 cows. These calculations are based on ranges for the cost of medicated mineral ($0.13 to $0.55/head/d), vaccination ($8 to $10/individual dose or $16 to $20 for the primary and booster) and serological testing ($6 to $9/head depending on which VDL you use, with a $2/head sample collection and processing fee).

The actual dollar figure will likely vary based on your location. Assuming that your producer is willing to blood sample the entire herd and pay for testing, knowledge of the seroprevalence of individual animals could allow implementation of a single or combinations of the following control strategies based on the overall production goals of the rancher:

- Targeted culling of positive cattle if the herd goal is disease eradication. These are typically seedstock producers or producers in non-endemic areas with a relatively low prevalence of disease.
- Targeted chemosterilization of positive cattle with oxytetracycline injections or imidocarb dipropionate. This is a strategy typically adopted by seedstock producers for high genetic merit cattle or for those "special" cows or herd bulls.
- Targeted and strategic feeding of CTC to control active infections. This assumes that mineral consumption will be consistent during the feeding period.
- Targeted vaccination of only negative cattle. In the absence of data to support vaccination, I am reluctant to recommend this course of action, but because testing is cheaper than the cost of the vaccine, only vaccinating negative cows may be more cost effective for a producer than blanket vaccination over several years.

It is important to recognize that anaplasmosis control programs that are predicated on the outcome of serological testing are based on the following assumptions:

1. Seropositive cows are persistently infected anaplasmosis carrier cows that are immune to reinfection. We believe that these cows may die from clinical disease if they are immunosuppressed and unable to control the emergence of new antigenic variants, but in general, these animals will likely not benefit from vaccination or CTC therapy once they recover from acute infection and the carrier state has become established.
2. The cELISA test for anaplasmosis is reliable. We know that in low prevalence areas, the test may cross-react with maltose-binding peptide in the serum of approximately 40% of healthy cattle but the test is typically considered to have a greater than 90% specificity and sensitivity in most

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herds. We typically recommend following up with targeted individual or pooled PCR testing if the risk of a false negative (resulting in disease persistence in the herd) or a false positive (resulting in culling of a high-dollar animal) would have significant consequence for the producer.

3. Calves under 6 months of age may have circulating maternal antibodies or may be disease-positive following in-utero infection and should be retested using either the cELISA test the following year or using an individual or pooled PCR test.
4. The producer maintains a closed herd and commits to testing all incoming animals.
5. The producer practices good biosecurity to minimize disease introduction and iatrogenic disease transmission through contaminated equipment during processing and vaccination.

Similarly, anaplasmosis control programs based on vaccination assume that the vaccine is effective and control programs based on CTC assume that intakes will be adequate and consistent across the herd and that the infection is susceptible to CTC. Unfortunately, there are no perfect options available but, in my opinion, knowledge of the herd disease status prior to implementing a disease control program is the most science-based approach available at this time.

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