Bovine anaplasmosis: What we know that just ain't so!

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Introduction

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 (US). 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.[5] 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.[1] Furthermore, a study has shown that 16% of pregnant carrier cows will transmit anaplasmosis *in utero* producing persistently infected offspring.[5] 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 US has been conservatively estimated at more than $400 per animal, with some estimating the total cost to the beef industry at more than $300 million per year.

1. Animals that Recover from the Infection are Free of the Disease

Anaplasmosis is one of the most challenging diseases facing cattle producers worldwide. After infection, there is typically a 4- to 8-week incubation period before clinical signs are observed (Figure 1).[4] 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. Cattle that recover from acute anaplasmosis maintain a microscopically undetectable parasitemia for life. Persistent infection is characterized by sequential rickettsemic cycles ranging from 10^2 to 10^7 that occur at about 5-week intervals.[3] 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, such as deer, and spread

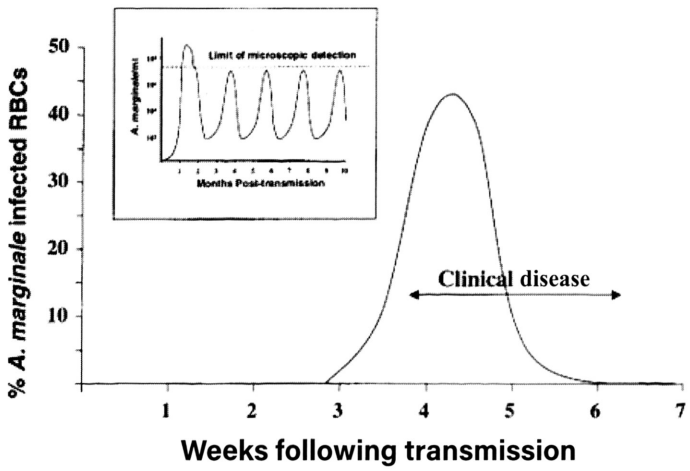

Figure 1.

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 treat acute anaplasmosis in the US. Therefore, it is critical that their efficacy be preserved.[2,5]

In addition to the costs associated with clinical anaplasmosis, animals recovering from acute anaplasmosis, including those treated with recommended doses of tetracyclines, remain lifelong *Am* carriers.[2] There are currently no antimicrobial compounds approved for elimination of persistent *Am* 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. This restricts the export of cattle from endemic areas such as the US 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, inadequate information regarding the usefulness of newer diagnostic tests in determining the success of disease eradication, and ineffective vaccines to protect against new infections.

2. Test-positive Animals are Infected, Test-negative Animals are not

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 AM in experimentally infected steers (Figure 2).[2] Forty Angus X Simmental steers were experimentally infected with 2.6 x 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 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 *Am*. However, it is 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 *Am* 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 *Am* 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.

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 University. 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 University are focused on developing a highly sensitive and specific diplex, RNA-based PCR diagnostic tool for identification of both *Am* 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 and others report a sensitivity of 30 infected erythrocytes per milliliter of blood for the DNA-based PCR.[9] 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.

3. All Cases of Anaplasmosis are Due to Transmission by Ticks and Biting Flies

The significance of iatrogenic transmission has recently been demonstrated in 2 studies. This study compared iatrogenic transmission of AM 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-

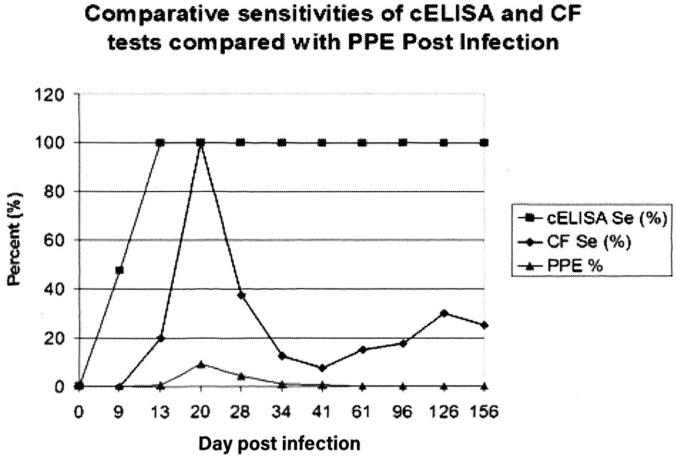

Figure 2.

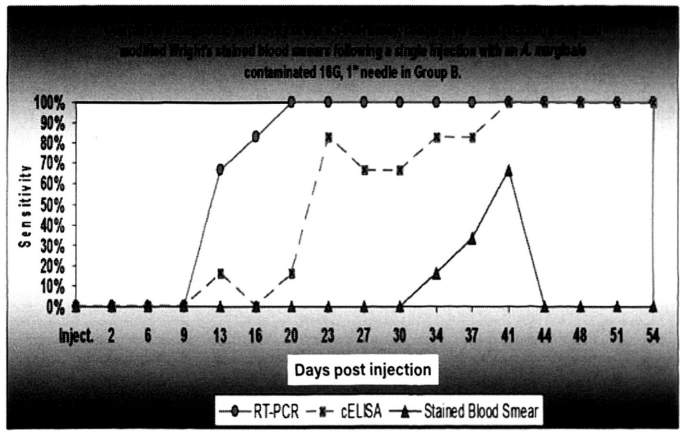

Figure 3.

six Holstein steers confirmed negative for anaplasmosis by cELISA and RT-PCR were infected with a Virginia isolate of *Am* 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-day 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 days 41, 41, and 20 post-vaccination, 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 *Am*. The sensitivity of cELISA and RT-PCR were similar following the acute phase of infection.

4. Persistent Anaplasmosis Infections Cannot be Cured

Chlortetracycline (CTC) and oxytetracycline (OTC) are the only compounds approved for use against acute anaplasmosis in the US. In regard to the oral administration of OTC or CTC, there are currently no compounds approved for the elimination of the carrier state in the US. Current label claims for CTC (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: - CTC 1 mg/lb (2.2mg/kg) orally daily for 41 days, CTC 0.5 mg/lb (1.1 mg/kg) orally for 120 days.

Chemosterilization has been reported in cattle fed CTC at dosages ranging from 0.5 mg/lb (1.1 mg/kg) for 120 days to 5 mg/lb (11 mg/kg) for 30 to 60 days. The relationship between plasma CTC drug concentration and carrier clearance has not been described until recently. In a study conducted by our research group, chronic carrier status was established in 21 steers with a Virginia isolate of Am and confirmed by cELISA and the previously described A. marginale-specific RT-PCR.8 Four naïve, splenectomized steers served as active disease transmission sentinels. Steers were randomized to receive either 2 mg/lb (4.4 mg/kg)/day (LD); 5 mg/lb (11 mg/kg)/day (MD); or 10 mg/lb (22 mg/kg)/day (HD) of oral CTC; or placebo (CONTROL) for 80 days. The LD, MD, and HD treatment groups consisted of 5 infected steers and 1 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%) CTC 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 53 of treatment. A negative RT-PCR assay result was confirmed in all CTC-treated groups within 49 days of treatment; however, cELISA required an additional 49 to 88 days before similar results. Subinoculation of splenectomized steers confirmed chemosterilization. These results demonstrate that CTC may be used to eliminate persistent *Am* infections, but cattle are susceptible to reinfection with anaplasmosis after clearance. This data is important for influencing future chemosterilization strategies and impacting free-trade policy among countries and regions of contrasting endemicity.

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 is a terminal producer. When a large number of cattle are dying from anaplasmosis, I would recommend mass-medicating with long-acting tetracycline, then pulse-feed CTC at 0.9 mg/lb (2 mg/kg) bodyweight/day for 30 days, take a 30-day break, then pulse-feed again for 30 days throughout the vector season. I would not recommend continuous feeding of CTC because I am concerned that we may be inadvertently chemosterilizing those cattle, making them completely susceptible to reinfection with anaplasmosis in subsequent seasons. Endemic instability in an endemic area could potentially be catastrophic for a producer.

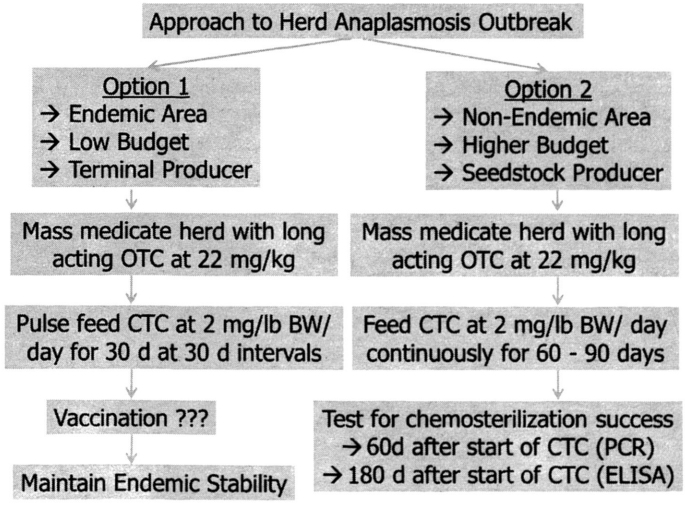

Figure 4.

The other option is vaccination. There are currently no USDA-approved vaccines to prevent anaplasmosis in cattle, so you would need to obtain conditional USDA approval to use the vaccines available, especially in certain states. Current vaccines may prevent animals from dying, but these do not prevent cattle from becoming carriers. If your client is a purebred 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, it is noteworthy that the vaccine only contains 1 isolate of anaplasmosis and thus may not cross-protect between all strains. In the absence of peer-reviewed data regarding the effectiveness of the vaccine, I am reluctant to recommend its use. However, this may be something a producer can consider if other control measures are unsuccessful.

Pulse feeding CTC is what I would recommend to maintain that endemic stability, but also to control the organism sufficiently to prevent mortality over the vector season. Producers in non-endemic areas, with a higher budget, may consider mass-medicating with injectable, long-acting OTC at 10 mg/lb (22 mg/kg) and then feeding CTC at 2 mg/lb (4.4 mg/kg) bodyweight/day continuously for 60 to 90 days (Option 2). A follow-up PCR test can then be used to determine if chemosterilizating was successful at 60 days after start of CTC, or the cELISA test could be used at 120 days after the start of CTC treatment.

There are several potential reasons why chemosterilization may be unsuccessful. The most common cause of unsuccessful chemosterilization is inadequate drug intakes when these compounds are administered orally. Personally, I don't believe a medicated mineral constitutes an adequate means of delivering CTC for anaplasmosis control in many regions, and studies are underway to confirm this. Chemosterilized cows could also become re-infected after treatment, and that could result in perceived treatment failures. Studies to investigate if antimicrobial resistance to tetracyclines could also contribute to perceived treatment failure are also underway.

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