PEER REVIEWED

Current challenges in the diagnosis and control of bovine anaplasmosis

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Abstract

Bovine anaplasmosis, caused by the intracellular rickettsia *Anaplasma marginale,* continues to impact cattle production in the US. Control and management of bovine anaplasmosis is influenced by our understanding of the complexity of this pathogen, interactions with both cattle and tick hosts, and the disease. *A. marginale* is maintained in nature through biological transmission by ticks and mechanical transmission by any means of blood transfer from infected to susceptible cattle. The major surface protein 5 (MSP5)-based cELISA, approved for use in the US and Canada, has proven to be a sensitive serologic test. However, more recently this test has been shown to be cross-reactive with closely related organisms, including *A. phagocytophilum*, because of the conservation of MSP5. Therefore, additional molecular tests may be required in some situations to confirm the identity of the infective agent. While tetracycline antimicrobials are approved by the US Food and Drug Administration for prevention or treatment of acute anaplasmosis, no antimicrobials are labeled for elimination of persistent *A. marginale* infection. Administration of tetracyclines is an important means of preventing clinical anaplasmosis, but does not prevent cattle from becoming infected with *A. marginale*. The mode of action of tetracycline is bacteriostatic rather than bactericidal, and its long-term use does not consistently clear cattle of persistent *A. marginale* infections. USDA approved vaccines for anaplasmosis are currently unavailable. The challenges of vaccine development include the antigenic variation of *A. marginale* that occurs during persistent infections and the increased antigenic diversity of *A. marginale* strains, especially in areas of ongoing cattle movement. The overall goal of this and a previous overview is to summarize the current status of knowledge and research on bovine anaplasmosis, and to provide veterinarians with answers to frequently asked questions.

Key words: bovine anaplasmosis, *Anaplasma marginale*, vaccines, treatment, chemotherapy, ticks

Résumé

L'anaplasmose bovine, causée par la rickettsie intracellulaire Anaplasma marginale, continue d'affecter les élevages bovins aux États-Unis. Nos méthodes de lutte et de gestion de l'anaplasmose bovine reflètent notre compréhension de la complexité de l'agent pathogène, des interactions entre les bovins affectés ou avec les hôtes de la tique et de la maladie. A. marginale se propage dans la nature d'un bovin infecté à un bovin sensible par l'intermédiaire des tiques et par tout autre moyen de transmission sanguine.

La technique cELISA pour détecter la protéine de surface majeure 5 (MSP5), approuvée aux États-Unis et au Canada, est un test sérologique sensible de détection de cet agent pathogène. Toutefois ce test s'est récemment montré sensible également dans la détection de microorganismes étroitement apparentés, tels Anaplasma phagocytophilum, en raison de la généralisation de la MSP5 parmi les organismes du genre Anaplasma. C'est pourquoi des tests moléculaires additionnels sont parfois requis pour confirmer l'identité de l'agent infectieux.

Bien que des antimicrobiens à base de tétracycline soient approuvés aux États-Unis par la Food and Drug

Administration pour la prévention ou le traitement de l'anaplasmose aiguë, il n'existe aucun antimicrobien approuvé pour l'élimination de l'infection persistante par A. marginale. L'administration de la tétracycline est une méthode largement utilisée pour prévenir l'anaplasmose clinique, mais elle n'empêche pas l'infection des bovins par son agent causal, A. marginale. En effet, la tétracycline a une action davantage bactériostatique que bactéricide et son usage prolongé ne débarrasse pas les bovins de l'infection persistante par A. marginale. Il n'existe pour le moment aucun vaccin contre l'anaplasmose approuvé par le USDA. Les chercheurs ont le double défi de trouver un vaccin antigénique contre la forme d'A. marginale qui cause l'infection persistante et contre les souches d'une diversité croissante de ce microorganisme retrouvées dans les lieux où transitent continuellement les bovins. La présente synthèse, tout comme celle effectuée précédemment, se veut un sommaire de nos connaissances actuelles et des recherches en cours sur l'anaplasmose bovine, et vise à répondre aux questions fréquemment posées aux vétérinaires.

Introduction

Bovine anaplasmosis, caused by the intraerythrocytic rickettsia *Anaplasma marginale*, is enzootic in the United States (US) throughout the southern Atlantic states, Gulf Coast states, and several midwestern and western states (as reviewed by Kocan *et al*[38,39,40]). The disease has been reported in all states of the US except Alaska and Hawaii. This widespread and apparently increasing distribution of *A. marginale* has likely resulted from unrestricted transport of asymptomatic but persistently infected cattle, which serve as a source of infection for biological transmission by ticks or mechanical and iatrogenic transmission. Because anaplasmosis is not a reportable disease in most states, its economic impact on US cattle production has been difficult to assess, and without this data companies and federal agencies have been less likely to invest in research and development of control measures. While selected wild ruminants become infected and serve as reservoirs of *A. marginale*, clinical disease occurs predominantly in cattle and persistently infected cattle serve as the major reservoir host.

Anaplasmosis is a herd problem. When clinical disease is observed in a herd, other cattle will likely be in the incubation phase, and therefore would require intervention to prevent clinical cases of disease. 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, infection can be transmitted *in utero* from dam to calf, resulting in the birth of infected but otherwise healthy calves. However, the overall occurrence of transplacental transmission has not been well documented in the US.[3,39,48,59,60] In infected cattle, a balance exists between the pathogen and the host immune response, and stressors such as extreme weather conditions, poor nutritional status, parturition, lactation periods, and concurrent infections may contribute to outbreaks of clinical anaplasmosis.

The primary site of *A. marginale* replication in cattle is within inclusion bodies in erythrocytes. The incubation period of infection (prepatent period) varies with the infective dose and can range from seven to 60 days, with an average prepatent period being approximately one month. High percentages of erythrocytes become infected during acute infection, and removal of infected ones by the bovine reticuloendothelial system approximately correlates with the severity of anemia and icterus.[52]

Clinical signs of bovine anaplasmosis are highly variable and range from subclinical persistent infections to severe peracute disease associated with significant production losses, abortions, and mortalities.[39] The acute phase of the disease is characterized in adult naïve cattle by severe anemia, weight loss, fever, abortion, lowered milk production, and death. Tentative diagnosis of bovine anaplasmosis can be made on the basis of historical information, including geographic location and season, and clinical signs or necropsy findings,[39] with subsequent confirmation by demonstration of inclusion bodies in stained blood smears and/or by use of serologic/molecular diagnostic assays (as reviewed in Kocan *et al*.[38,39]

The dynamics of *A. marginale* transmission are more complex than other tick-borne diseases of cattle because transmission occurs in two main routes: 1) biologically by ticks, where *A. marginale* infects and multiplies in ticks and then is transmitted to susceptible cattle during tick feeding and/or 2) mechanically or iatrogenically by any means of transfer of infective blood, including a wide variety of blood-contaminated fomites such as veterinary instruments, hypodermic needles or biting flies.[38,39] Following acute disease, *A. marginale* establishes lifelong persistent infections in cattle, characterized by low-level (10^2–10^7 parasitized erythrocytes) sequential rickettsemic cycles that occur at approximately five to six-week intervals.[35] During each cycle, an antigenic variant arises as a result of combinatorial gene conversion by the immunodominant outer membrane protein gene families *msp2* and *msp3*.[9] Infection with these variants can result in individual clinical cases despite the use of killed vaccines, and therefore may raise concern regarding vaccine efficacy. Maintenance of persistent infections confers lifelong immunity to the host and contributes to stability of anaplasmosis in endemic areas. Exposure of calves results in development of persistent infections without clinical disease. In non-endemic areas, focal outbreaks in susceptible cattle occur most often by mechanical

transmission from imported cattle that are persistently infected, but otherwise healthy.

Control options for anaplasmosis vary depending upon whether outbreaks occur in a known endemic area or as focal isolated events in non-endemic areas, and include 1) maintenance of an *A. marginale*-free herd, 2) vaccination, 3) treatment with tetracyclines during acute disease, and 4) administration of low-level tetracycline for prevention of clinical disease (as reviewed by Kocan *et al*[39]). Importantly, it should be noted that the goal of incorporating vaccines or antimicrobials in preventive health protocols is the prevention of clinical anaplasmosis, and neither treatment prevents infection of cattle after challenge exposure. Cattle used as seed stock, including cows that are used only as embryo donors and bulls for semen collection or are exported to non-endemic areas, should be completely free of *A. marginale* infection. In areas where persistently-infected cattle are frequent and contribute to endemic stability, it may be important to maintain this situation rather than to introduce susceptible cattle which will likely result in the risk of development of acute disease. In some areas, especially in southern regions where tick vectors are frequently active during warm winters, control strategies may be required year round, while in other areas control may be required primarily during the vector season plus one month (accounting for the incubation period). Burning pastures had been suggested as a control method to reduce tick populations. However, the effect of burning on tick populations may be temporary because deer and other wild animals, which are major hosts for ticks, may carry ticks back into burned areas. Nonetheless, ticks cannot immediately re-establish their populations due to the lack of hospitable microhabitats. Current research at Oklahoma State University suggests that long-term application of controlled burning regimens reduces tick abundance in pastures, lowers the number of ticks on infested cattle, and increases mortality of ticks in the most recently burned pastures (Polito and Reichard, personal communication). Control of tick infestations by vaccination may also be an option to reduce anaplasmosis prevalence in tropical and subtropical regions.[14]

Factors that Impact Diagnosis and Control of Bovine Anaplasmosis

Despite research conducted over the past five decades, control strategies for bovine anaplasmosis have advanced minimally since the first anaplasmosis vaccine that was marketed in the 1960s. However, research has contributed to our understanding of the complexity of *A. marginale* and bovine anaplasmosis (as reviewed by Kocan *et al*,[38,39] Aubry[3]). While this review is focused on diagnosis and control of bovine anaplasmosis, we briefly address several aspects of bovine anaplasmosis because of their importance to the design and application of diagnostic and control programs.

Diversity of *A. marginale* Strains or Genotypes

Research conducted since 2000 has demonstrated a much greater diversity of *A. marginale* strains or genotypes than recognized previously.[2,4,20,21,22,23,24,45,53] This diversity has become apparent, in part, because molecular tools are now available for strain definition by sequence of the *A. marginale* major surface proteins (MSPs) and other gene sequences. While MSP1a is a stable marker of strain identity, phylogenetic analysis of MSP1a does not provide information of the geographic origin. However, MSP4 sequences provide both strain identity and phylogeographic information.[19,25] The overall result of these studies is that the diversity of *A. marginale* strains is extensive, especially in areas of intense cattle movement, and individual strains continue to be maintained in the cattle population by independent transmission events. This increased strain diversity complicates control strategies because widely diverse strains may not be cross-protective when used as vaccine antigen(s). Additionally, *A. marginale* strains may not have uniform susceptibility to antimicrobials (Coetzee H., unpublished data).

Research has clearly demonstrated that some strains of *A. marginale* are not transmissible by ticks. For example, strains of *A. marginale* from Florida were found not to be infective for ticks.[17,56] Therefore, outbreaks of anaplasmosis in some areas of Florida most likely resulted from mechanical transmission, rendering tick control an unnecessary component of a control program in this region.

Maintenance of *A. marginale* Strains by Infection-Exclusion

Recent research demonstrated that maintenance of different genotypes can occur in nature because of a mechanism of infection-exclusion of *A. marginale* in cattle and ticks. In infection-exclusion, the establishment of one *A. marginale* genotype prevents a second genotype from becoming established after challenge exposure.[5,15,16] However, subsequent research demonstrated that a low percentage of cattle could become infected with more than one *A. marginale* strain when the strains are not closely related.[47] Overall, the importance of these findings helps to explain the mechanism by which multiple *A. marginale* strains occur and are maintained within a herd of cattle.

Transmission of *A. marginale*

For optimum control of anaplasmosis, it is fundamental to determine whether outbreaks occur in an

endemic area or as focal occurrences in non-endemic areas. In endemic areas, all three means of transmission (biological, mechanical (including iatrogenic), and transplacental) are likely to occur and must be considered during development of control programs. When cattle become infected as calves, they develop persistent infections without clinical signs and contribute to endemic stability. In contrast, sporadic outbreaks in non-endemic areas are most likely to occur in cattle over two years of age, and most often result from mechanical transmission from imported persistently infected carrier cattle. Changing distributions of tick populations may also contribute to anaplasmosis outbreaks and establishment of endemic anaplasmosis in new areas of the US. Male ticks have more recently been identified as the major tick developmental stage for biological transmission of *A. marginale* because male ticks become persistently infected, are intermittent feeders, and transfer readily among cattle within a herd.[41,42] Because of the repeated feedings on multiple cattle, the male tick is the stage of concern when formulating control strategies. These persistently-infected male ticks have life-long infections, and therefore also serve as a reservoir of *A. marginale.* Male ticks are most likely important in the dynamics of *A. marginale* transmission by one-host ticks, such as *Rhipicephalus* (formerly *Boophilus*) spp and *Dermacentor albipictus*, by being the tick stage that transfers among cattle.[41,42]

Iatrogenic (mechanical) transmission of *A. marginale* infection occurs when a common needle is used successively on cattle during treatment and vaccination practices or when veterinary instruments are not sterilized between animals. Mechanical transmission also commonly occurs from infected to susceptible cattle after blood transfer by biting insects, most notably tabanids (horse and stable flies).

Finally, transplacental or *in utero* transmission has been found to occur more frequently than previously recognized, and 16 to 20% of infected calves in a herd may be born from infected dams. While these infected calves are apparently healthy, they are and will remain lifelong, persistently-infected carriers.

Persistent Infections in Cattle and Ticks

All cattle that become infected with *A. marginale* (calves from *in utero* or mechanical transmission or adults after acute infection) remain persistently infected for life.[28,29,35,48] Persistently infected cattle serve as reservoirs in nature, thereby being a source of infection for mechanical transmission by blood-contaminated fomites and biting flies, and for biological transmission by ticks. Clearance of *A. marginale* infections in cattle is difficult to unequivocally confirm. More importantly, if cattle are cleared of infection by prolonged exposure to tetracycline, they will become fully susceptible to re-infection.

Antigenic Variation in Persistently Infected Cattle and Ticks

Persistently infected cattle undergo five- to six-week cycles in which an immune response is mounted to a circulating *A. marginale* variant, followed by clearance of the variant.[28,29,35] Subsequently, a new population of the next antigenic variant arises, creating another cycle of infection followed by immune control. Efficacy of vaccines or antimicrobials may be compromised by these constantly changing variants because of differences in treatment susceptibility.

Male ticks can persist in the environment, and *A. marginale* in these persistently-infected ticks also undergo antigenic variation.[18,41] Transmission of these variants to susceptible cattle could contribute to the overall antigenic diversity of *A. marginale* in any given region.

Stress

Stressors such as poor nutrition, adverse weather conditions, calving, lactation, and concurrent infections contribute to development of clinical disease. Acute anaplasmosis results from increases in infected erythrocytes followed by removal, and elevated parasitemia would contribute to enhanced mechanical transmission and infection and biological transmission by ticks. Cattle are often infected with multiple hemoparasites, including *Mycoplasma wenyonii*, *Bartonella* spp, *Theileria* spp, and *Trypanosoma* spp, which normally do not cause clinical disease, but concurrent infections could result in immunosuppression and compromise the bovine immune response to *A. marginale*, which is important for maintenance of persistent infections.

Emerging *Anaplasma* spp

An emerging tick-borne disease was first described in the north central US as human granulocytic ehrlichiosis (HGE)[31] (as reviewed by Woldehewit[57]). The etiologic agent, *A. phagocytophilum*, was named after a comprehensive reclassification of the family Anaplasmataceae, and the associated disease in humans is now known as human granulocytic anaplasmosis (HGA).[27] Previously, the host range of organisms of the genus *Anaplasma* was limited to ruminants. For example, while bison serve as a reservoir of *A. marginale,* this pathogen primarily infects and causes disease in cattle. The less pathogenic *A. centrale*, used as a live vaccine in parts of Europe and Africa, is largely limited to cattle and presently is not found in the US. *A. ovis* is primarily host-specific for sheep and goats, and infections have not been described

in cattle. In contrast, *A. phagocytophilum* has a wide host range, including humans, ruminants, small mammals, cats, dogs, horses, and birds, with some genotypes being more host-specific, and has been reported in cattle in several European countries.[22,32,57] Since this pathogen is emerging in the animal and human populations of the US, it may in the future be recognized as a pathogen of cattle. While *A. phagocytophilum* has been shown experimentally to infect cattle, the pathogen has not yet been reported in the US cattle population. If cattle become infected with *A. phagocytophilum*, they would likely test positive by the MSP5 cELISA. Therefore, a molecular diagnostic test will be required for differentiation between *A. marginale* and *A. phagocytophilum*.[26]

In summary, all of the factors reviewed and discussed herein contribute to the complexity of bovine anaplasmosis. The impacts of these factors on the diagnosis and control of bovine anaplasmosis using antimicrobial therapy and vaccination are addressed in the following sections on diagnosis, treatment, and control.

The Current Status of Diagnosis of Bovine Anaplasmosis

The "gold standard" for diagnosis of *A. marginale* in cattle is the inoculation of a suspect blood sample into a susceptible, splenectomized calf. While intact calves are susceptible to *A. marginale* infection, they rarely develop clinical signs of acute disease. However, calves are fully susceptible if splenectomized to challenge-exposure with infective blood.[34] While the use of this gold standard is an important component of the development of diagnostic tests and strategies for chemosterilization, the use of this approach is obviously impractical for large-scale use in cattle.

Tentative diagnosis of bovine anaplasmosis can be made based on a combination of the geographic location, season, clinical signs and/or necropsy findings (as reviewed by Kocan *et al*[39]). Demonstration of *A. marginale* inclusion bodies in stained blood smears can also be used for diagnosis, but may not be reliable when parasitemias are low prior to clinical signs or in persistently-infected carrier cattle. During these times *A. marginale* inclusion bodies can be easily confused with Howell-Jolly bodies, basophilic stippling of immature erythrocytes, and stain contamination.

Serologic tests have been developed for diagnosis of anaplasmosis and, more recently, a variety of molecular diagnostic assays have also been formulated and tested. Serologic assays are currently the most cost-effective way to screen large numbers of cattle for *A. marginale* infection. The serologic tests developed initially for diagnosis of anaplasmosis, the complement fixation (CF) and card agglutination (CA) tests, were useful for identification of acutely infected cattle.[13] While these tests had high specificity, they proved to have low sensitivity. The CF and CA tests were not effective for diagnosing cattle during the prepatent or incubation periods and during persistent infections.[7,13] In addition, these early serologic tests were developed and tested using one isolate, and test performance was not evaluated using genetically diverse *A. marginale* strains.

A competitive ELISA (cELISA) based on the *A. marginale* MSP5 surface-exposed protein, developed in the mid-1990s, proved to be far superior to previous serologic tests (sensitivity, 96% and specificity, 95%). The cELISA was effectively used to identify most infected cattle during prepatent periods and persistent infections.[13,36] The cELISA is practical and cost-effective for screening serum collected from large numbers of cattle, and this test has become the USDA-approved test for detection of anaplasmosis in the US and Canada. However, expanded use of this test has included false positives, especially during the prepatent period of infection.[30] Recently, maltose-binding protein (MBP) binders, found in approximately 40% of bovine sera tested, may have been the cause of the false-positive results because MBP is present in MSP5 recombinant antigen constructs.[11] The inclusion of a MBP absorption procedure in the current assay was found to improve the diagnostic specificity of the cELISA test.

Once blood samples have been collected from a persistently infected animal of a known seropositive status, polymerase chain reaction (PCR) assay results are accurate (sensitive) up to 120 hours in samples stored under ambient or refrigerated conditions (UV light exposure not allowed during either storage condition). In addition, postmortem assay results using samples collected from large blood vessels of persistently infected animals are accurate for up to 12 hours after death, but may not be relevant to the cause of death (Reinbold and Coetzee, unpublished data).

DNA- and RNA-based PCR assays based on highly conserved *A. marginale* genes have been developed over the past 20 years.[51] PCR assays are more sensitive for detection of organismal-specific DNA or RNA, and therefore are able to identify and confirm *A. marginale* infection. Due to the time and expense of these tests, PCR assays are not practical for large scale surveillance of *A. marginale* infection in cattle, but they are useful as a definitive diagnostic tool when serologic results require confirmation by providing organism and strain identity. For example, the cELISA recently demonstrated false positives in a herd of cattle in British Columbia where the disease had not been reported previously during a routine surveillance for anaplasmosis.[33] PCR studies using the *A. marginale msp5* and rickettsial 16S ribosomal RNA gene sequences subsequently demonstrated that these cattle were infected with a novel *Erhlichia* rather than *A. marginale*.[31]

A complication recognized with the MSP5 cELISA is that this test cross reacts with antibodies to organisms of the genus *Anaplasma*.[26,55] The *msp5* gene is highly conserved among the *Anaplasma*, therefore rendering this test accurate to the genus but not the species level, and this fact should be taken into consideration when evaluating the research literature prior to 2000. For example, studies based on the cELISA reported that mule deer were reservoirs of *A. marginale*; however, more recent PCR studies confirmed that the mule deer were infected with *A. ovis*.[19,58]. The MSP5 cELISA is also cross-reactive with sera from animals infected with *A. phagocytophilum*. In a recent study in which a sheep model was developed for a human isolate of *A. phagocytophilum,* serum samples from all experimental sheep tested positive by use of this MSP5 cELISA.[37] Because of this cross reaction the possible emergence of *A. phagocytophilum* in the US cattle population would not be detected and differentiated from *A. marginale* infection by use of the cELISA.

An RNA-based real time RT-PCR assay has recently been developed that differentiates between the two organisms in cattle sera,[51] but this test currently has not been widely adapted for use in diagnostic laboratories for the routine screening of bovine blood samples. When cELISA results in cattle are positive in the absence of clinical disease or a history of endemic anaplasmosis, cattle should be tested by PCR to either confirm *A. marginale* infection or determine the infective agent that caused the positive results.

The Current Status of Chemotherapy for Ttreatment and Control of Bovine Anaplasmosis

Tetracyclines are labeled by the US Food and Drug Administration (FDA) for the treatment of bovine anaplasmosis in the US,[6] and the overall goal of their use in control programs is for prevention of clinical signs. Tetracyclines are bacteriostatic drugs that function by binding to ribosomes and mRNA. The resulting inhibition of bacterial protein synthesis is mediated principally through reversible binding with the 30S ribosomal subunit.[10,54] This feature may explain why the bovine response to treatment with tetracyclines appears to be time-dependent as opposed to concentration dependent.[49,50]

In the absence of an approved anaplasmosis vaccine for cattle, tetracyclines represent the major means of control for bovine anaplasmosis in the US. The two forms of tetracycline for anaplasmosis control are chlortetracycline (CTC) as a feed supplement and oxytetracycline (OTC) as an injectable. It should be noted that feed additives used off-label are illegal in food-producing animals in the US. Current label claims for CTC[a] are as follows:

- **Beef cattle (over 700 lb; 318 kg):** Control of active infection of anaplasmosis caused by *Anaplasma marginale* susceptible to CTC at a dose of 0.5 mg/lb (1.1 mg/kg) CTC body weight/day.
- **Beef and non-lactating dairy cattle (over 700 lb; 318 kg):** 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 at a dose of 0.5 to 2.0 mg/lb (1.1 to 4.4 mg/kg) CTC body weight/day.

Published studies that claim clearance of carrier infections used the following variations of labeled oral dose regimens: 1 mg/lb (2.2 mg/kg) CTC daily for 41 days or 0.5 mg/lb (1.1 mg/kg) CTC 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. Many previous studies that claimed clearance of persistent *A. marginale* infections in cattle using OTC or CTC were based on serologic tests which lacked the sensitivity for detection of low-level persistent infections.[7] Chemosterilization studies recently repeated using the currently recommended World Organization for Animal Health, Office International des Epizooties (OIE)[46] treatment protocol of five daily injections of oxytetracycline administered intravenously at 10 mg/lb (22 mg/kg) failed to eliminate persistent *A. marginale* infections.[12] Importantly, a reliable treatment regime for elimination of persistent *A. marginale* infection in cattle is currently unavailable. Therefore, transport of cattle from endemic to non-endemic areas should be avoided to prevent introduction of *A. marginale* infection and disease in populations of susceptible cattle.

Recently, CTC fed at 5 mg/lb (11 mg/kg)/day or 10 mg/lb (22 mg/kg)/day to feedlot steers persistently infected with anaplasmosis reportedly resulted in negative RT-PCR and cELISA tests 49 days and four to six months, respectively, after treatment commenced.[50] Chemosterilization was confirmed by subinoculation of blood from each treated animal into a splenectomized calf 50 days after the 80-day treatment period. It should be noted that only the 2.0 mg/lb (4.4 mg/kg)/day dose of CTC is legally permissible for control of active *A. marginale* infections under the Animal Medicinal Drug Use Clarification Act and that in this study, plasma CTC concentrations were maintained at steady state for 70 to 72 days. Importantly, chemosterilized calves were shown to be completely susceptible to re-infection when challenge-exposed with the same *A. marginale* isolate after chemosterilization.

Based on these findings, CTC as a feed supplement at 2 mg/lb/day (4.4 mg/kg/day) for 30 days is

recommended for use on a herd basis to control active infections in endemic areas. Longer periods are not recommended because cattle could possibly become cleared of *A. marginale,* and would then be fully susceptible to re-infection when challenge-exposed by mechanical or biological transmission.[50] Presently, the best strategy in endemic areas is the use of CTC pulse feeding for 30-day periods, although further research is urgently needed to confirm the effectiveness of this approach. A risk of administering CTC as a feed supplement is that all cattle may not consume sufficient amounts of feed to maintain sufficient plasma drug concentrations for periods long enough to eliminate persistent infections. This is especially the case when CTC is delivered in mineral supplements because consumption may vary greatly among individual animals and seasons. While earlier reports suggested that *A. marginale* strains had uniform susceptibility to oxytetracycline,[43] more recent evidence suggests that differences in susceptibility may occur between *A. marginale* strains (Coetzee *et al*, unpublished data). While tetracycline resistant *A. marginale* strains have not been reported, the genome of *A. marginale* was found to contain sequences consistent with the presence of multi-drug resistance pumps.[8] While other antimicrobials, such as enrofloxacin, may show promise for treatment of anaplasmosis, the FDA prohibits the extra-label use of this drug in food producing animals, and the drug is not labeled for treatment or clearance of bovine anaplasmosis.

Administration of tetracyclines to cattle reduces *A. marginale* parasitemias and antibody levels, and during and shortly after treatment cattle could possibly become both serologically and RT-PCR negative while still being infected with *A. marginale* (Coetzee, unpublished data). Serologic testing of cattle for exportation should not be done until four to six months after the last exposure of cattle to chlortetracycline in order to increase the probability of an accurate test result. If chemosterilization is the desired treatment outcome, treatment should persist for at least an additional 30 days after the first negative RT-PCR assay result, and then be confirmed with two to three serial negative assay results done at 30-day intervals after the first negative PCR result (Coetzee, unpublished data).

Treatment of clinical cases of anaplasmosis should be considered with respect to the severity of the disease and logistics of handling the animal for treatment. The stress of handling cattle with clinical anaplasmosis, especially those with a hematocrit less than 10%, may result in sudden death because the animal is unable to compensate for lost oxygen carrying capacity and increased demand for oxygen at the tissue level. This may be exacerbated by the physical exertion of moving the animal to the processing facility. In addition, animals with clinical anaplasmosis may become aggressive, presumably due to cerebral anoxia and respiratory distress. Handling may exacerbate these problems.

In most cases the parasitemia in severely jaundiced animals has peaked, and may be resolving as a result of the host immune response. Therefore, administration of antimicrobials during the anemic crisis may not significantly alter the course of the disease. In contrast, cattle with rising parasitemia that are not severely compromised may benefit from a single IM or SQ injection of oxytetracycline (10-13.6 mg/lb; 22-30 mg/kg), provided the animal can be treated without stress. While distinguishing stages of clinical infection in the field may be challenging, administration of tetracycline is advocated provided this can be achieved safely and without causing additional distress to the animal.

Although IV injection of oxytetracycline would appear to be preferable because of the intra-erythrocytic location of the pathogen, published evidence has not been presented to suggest that IV administration produces a superior therapeutic outcome. In addition, a study of oral chlortetracycline demonstrated that the response to tetracycline therapy was independent of plasma drug concentrations, but related to the duration of exposure to the drug,[50] suggesting that concentrations achieved following IM or SC dosing should be as effective, if not superior, to IV dosing. It should also be noted that a single injection of oxytetracycline, irrespective of the route of administration, will not clear infection.

The perceived benefits of IV drug administration should also be weighed against the additional stress associated with the restraint required to achieve vascular access. Similarly, although blood transfusions may be indicated in valuable animals when the hematocrit is less than 15%, the stress on the compromised cardiovascular system may outweigh the benefits of the transfusion. Importantly, transfusion is also accompanied by the risk of exposing the animal to other hemoparasites and bovine leukosis virus.

Mass medication of the herd with parenteral oxytetracycline in the face of an anaplasmosis outbreak has been advocated. However, exposure to tetracycline during the prepatent period of the infection may simply prolong the onset of the disease without altering the course of the clinical infection. Although naïve cattle injected with a tetracycline may not acquire anaplasmosis infection, this effect is unlikely to extend significantly beyond the 5-7 day period of circulating tetracycline concentrations achieved after IM or SC injection of 10 mg/lb (22 mg/kg) of oxytetracycline. Therefore, additional supplementation with chlortetracycline in feed at 2 mg/lb (4.4 mg/kg) bodyweight/day is indicated. Clinical anaplasmosis is typically associated with rumen stasis and hepatic injury. Therefore supportive therapy with vitamin B injections or rumen stimulants may be indicated although evidence that these significantly

improve treatment success are deficient in the published literature.

A future concern about the use of tetracyclines as a major control method for bovine anaplasmosis is the possible restriction of the general use of tetracyclines in cattle as feed supplements because of the growing concern over development of overall drug resistance. In the absence of an efficacious vaccination or antimicrobial therapy, US cattle producers would be without an effective control method for bovine anaplasmosis.

The Current Status of Vaccines for Control of Bovine Anaplasmosis

Vaccination has been an economical and effective way to reduce clinical anaplasmosis in cattle.[38,39] Two major types of vaccines, killed and live, were marketed previously in the US, both of which relied on *A. marginale*-infected blood as the antigen/infection source. While vaccines induce production of antibodies that reduce or limit *A. marginale* infection levels and therefore clinical disease, there are two important considerations of killed vaccines. First, vaccinations (initial and booster, followed by a yearly booster) must be done prior to an outbreak or vector season to allow for development of the antibody response. Second, killed vaccines do not prevent *A. marginale* infection in cattle; immunized cattle are fully susceptible to *A. marginale* and will become persistently infected upon challenge exposure.

Killed vaccines developed in the US in the 1960s were marketed until 1999, when they were withdrawn from the marketplace due to company restructuring. A side effect of the first killed erythrocyte-derived vaccine was neonatal isoerythrolysis in calves born to dams vaccinated during pregnancy when antibodies were produced against the calf's blood type. Healthy calves that acquired these antibodies in the colostrum subsequently developed isohemolytic disease. Subsequent killed vaccines were produced to avoid this side effect by being purified to remove erythrocyte stroma. In addition, the vaccine was recommended not to be administered during pregnancy.

Currently, USDA-approved anaplasmosis vaccines are not commercially available in the US. A conditional killed vaccine made from one *A. marginale* strain (a Mississippi isolate) is currently being produced in Louisiana[b] and sold in approximately 17 states. A modified-live vaccine is commercially available for use only in California.[c]

As mentioned previously, the diversity of *A. marginale* strains in the US is much greater than previously suspected, especially in areas of extensive cattle movement, and these strains may not be widely cross-protective when used as vaccine antigens.[21,44] Therefore, by use of current technology, development of a killed vaccine protective against a wide range of strains may not prove feasible.

Live vaccines used in some European and African countries which involve infection of cattle with the less pathogenic *A. centrale* are not approved for use in the US. Cattle infected with the *A. centrale* live vaccine develop life-long persistent infection which provides protection against clinical disease when cattle are challenge-exposed with *A. marginale*. However, live vaccines depend on donor cattle as a source of infective blood, which could result in transmission of other hemoparasites including mycoplasmas, rickettsiae, and viruses carried silently by cattle.

The success of future vaccines for anaplasmosis using molecular technologies will depend on their ability to be cross-protective among genotypes and to either mimic, redirect the host response during natural infections and/or block infection of host cells. The ideal anaplasmosis vaccine would be one that induces protective immunity and prevents infection of both cattle and ticks, as well as abolishing the vectorial capacity of ticks. While much research has been done on the nature of the immune response of cattle to *A. marginale*, as well as identifying key *A. marginale* antigens that play a role in infection and the immune response, the challenges of strain diversity and antigenic variation of *A. marginale* in persistently infected cattle and ticks and the lack of industrial support and research funding do not suggest that development of a new or novel vaccine will occur in the near future.

Conclusions

Herein, we presented an update of the challenges inherent in the diagnosis and control of bovine anaplasmosis in the US. Research findings over the past five decades have contributed to our understanding of the increased complexity of disease and host-pathogen interactions. The diversity of *A. marginale* strains is much greater than previously appreciated, and increases the difficulty of developing vaccines cross-protective among these genetically diverse strains. Cattle and ticks become persistently infected with *A. marginale*, during which the pathogen undergoes antigenic variation which also increases the overall complexity of diagnosis and control. While the MSP5-based cELISA developed for diagnosis of anaplasmosis has been shown to be sensitive and specific for *A. marginale* antibodies and is approved for use in the US and Canada, this test has been shown more recently to be cross-reactive with other closely related pathogens due to the conservation of the *msp5* gene among *Anaplasma* spp. Therefore, subsequent testing of cattle with molecular tests may be needed to definitively identify the infecting pathogen.

The classification of *Anaplasma*, reorganized in 2001, now includes several additional organisms, including *A. phagocytophilum,* an emerging tick-borne pathogen in the US. While *A. phagocytophilum* has not been reported in the US cattle population thus far, concurrent infections of *A. marginale* and *A. phagocytophilum* have been demonstrated experimentally. *A. phagocytophilum* could possibly emerge in the cattle population in the future and impact cattle production. However, the MSP5-based cELISA is cross-reactive for *A. marginale* and *A. phagocytophilum*, and a positive cELISA in the absence of clinical signs or a history of bovine anaplasmosis may require subsequent molecular testing for confirmation of pathogen identity.

Currently, USDA approved anaplasmosis vaccines are unavailable in the US. However, a conditional vaccine made from one *A. marginale* strain (a Mississippi isolate) is available in Louisiana with limited geographic distribution. The genetic diversity of *A. marginale* strains in the US is much greater than suspected previously, especially where extensive cattle movement has occurred, and these strains may not be widely cross-protective when used as vaccine antigens. Therefore, development of a killed vaccine protective against a wide range of strains may be unfeasible at this time. Molecular approaches to vaccine development for anaplasmosis have not been reported, but molecular technologies are rapidly evolving and when coupled with information derived from genome sequences, may provide a new approach for development of a novel and effective vaccine.

Endnotes

[a]Aureomycin 90, Alpharma Animal Health, Bridgewater, NJ

[b]Anaplasmosis Vaccine, University Products LLC, Baton Rouge, LA

[c]AnaVac; PHL Associates, Davis, CA

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