Host, vector, environment and management: epidemiology of bovine anaplasmosis in the State of Kansas

by

Mark Randall Spare

B.S., Kansas State University, 2017
D.V.M., Kansas State University, 2019

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Diagnostic Medicine and Pathobiology
College of Veterinary Medicine

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Abstract

Bovine anaplasmosis was first described in the state of Kansas in 1926. Today, nearly a century later, this disease continues to impact beef cattle health having negative impacts on profitability and creating significant hurdles for genetic improvement in the cattle industry. The characteristics of *Anaplasma marginale* allow the bacterium to use unique opportunities within different environments to maintain infection presence and evade interventions. Extra-label uses of the antibiotic in animal feeds, including common dosages for the control of anaplasmosis, are illegal. Current management to control anaplasmosis in beef cattle herds is an integrated approach that requires decisions to be made at the individual animal and population herd level. The objectives of this dissertation were to describe the distribution and infection prevalence of the tick vectors and cattle populations at a similar point in time in Kansas to offer baseline information to aid producers and their veterinarians. Another goal of the dissertation was to examine the effects of approved therapeutic regimens on the clinical outcomes in naturally infected cattle in a commercial setting. In the first study, the questing tick vector was collected and identified over a two-year period in the Flint Hills ecoregion of Kansas. The association of the vector with climate data and land cover was modeled using general linear mixed modeling. The results of this study demonstrated significant associations between climate and tick density. In the second study, the infection prevalence of cattle throughout the state was described at the herd level and a survey administered to participating cattle operations. The results of this study demonstrated a widespread, but unequal herd-level infection prevalence across the state which is associated with specific management practices. The final study included in this dissertation was conducted to examine the effects of feeding an approved level of chlortetracycline medicated therapy, with or without supplemental injectable oxytetracycline, to chemosterilize yearling beef

bulls naturally infected with anaplasmosis in a commercial feeding situation. The results of the study indicate that the delivery of chlortetracycline for 80 days is insufficient to chemosterilize group fed yearling beef bulls. Injectable oxytetracycline in combination with chlortetracycline also had no effect on the chemosterilization of study cattle. All beef bulls naturally infected with anaplasmosis with lower level of reported inhibition percentage according to competitive enzyme linked immunosorbent assay were chemosterilized within 40 days on chlortetracycline, while no bulls with higher reported inhibition percentages were chemosterilized regardless of treatment. The research encompassed in this dissertation establish new baseline information for the distribution of bovine anaplasmosis within the vector and host populations throughout the state of Kansas. The inability for approved antibiotic therapy in a commercial setting adds to the understanding and expectations for producers and veterinarians for the use of this tool for the control and treatment of anaplasmosis in their herds. Further research is warranted in the prevention, control, and within herd epidemiology to guide future decisions in the management of this vector-borne disease in the state.

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Approved by:

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Dr. Daniel Thomson

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Dedication

What an end to a journey, and the start of a myriad of opportunities. It is with considerable reflection, appreciation, and admiration for those involved that I submit this dissertation for approval. To my dear Kayla, you alone know the challenges that you faced while I traveled for study days, spent evenings in class, and took days to sit in front of a computer screen. You have supported this endeavor with your strength, your patience with our girls, and your love which lasts above all. To my dear Elizabeth and Lydia, you will never know how much your Daddy wanted to hold you and be with you as you spent your days during the completion of this degree. You are a joy to be around, and an inspiration for me to push through each challenge, just as you both exemplify in your own lives. To my parents, who have walked with me as a young cowboy in Russia to a DVM/PhD student in Manhattan, KS. Your commitment to each other and to the relationships in your family and community drive me to be a better husband, father, and community member. To Dr. Martha Vanier, who shared office space at Horizon Ranch during the shutdown of 2020 and supported our family with friendship during our time in the Flint Hills. Your example of generosity and servant-leadership are much admired and will forever change me. Most importantly, I want to dedicate this dissertation to my Lord and Savior Jesus Christ, by whose sustaining grace I live and breathe and enjoy working with cattle, with horses, and the men and women who love and cultivate both of these.

Preface

Chapter 3 of this dissertation was prepared for and published in Veterinary Parasitology X in 2020 (Volume 3, May 2020). Chapter 4 of this dissertation were was prepared for submission for publication in Bovine Practitioner. These chapters have been edited to fit into a format consistent with this dissertation.

Chapter 1 - Bovine Anaplasmosis in Kansas: A review

Abstract

Bovine anaplasmosis is a tick-mediated hemolytic disease of cattle caused by the rickettsial bacterium *Anaplasma marginale*. Possibly the most widespread tick-mediated disease of cattle across the world, the bacterium respects neither border nor breed. The economic impact in the United States alone is estimated to cost livestock producers approximately $300 million annually. Clinical signs of the disease are fever, weight loss, aggressive behavior, abortion, respiratory distress, depression, and adult animal sudden death. Severe clinical signs most commonly manifest in cattle older than two years of age. Stress caused by restraint for the treatment of acutely infected animals can lead to death. Robust immune defense evasion mechanisms, multiple mechanisms of transmission, biological magnification, and routine mutations allow the pathogen to persist in a variety of regions and environments. Poor treatment success coupled with an estimated mortality of 30-50% of infections necessitate management and veterinary intervention in cow-herds to mitigate potential loss. Incomplete understanding of these characteristics prevent effective management by the livestock industry and veterinary profession. Further research is warranted regarding distribution and prevalence of infection in common vectors, infection prevalence of regional tick populations, and practical application of labeled treatments in commercial situations.

Introduction

Bovine anaplasmosis is a tick-mediated hemolytic disease of cattle caused by the rickettsial bacterium *Anaplasma marginale*. Estimated economic losses due to the disease range in the several millions of dollars every year in the US alone (Goodger et al, 1979). Previous estimates have indicated that a clinical case of bovine anaplasmosis costs roughly $400/animal

(Goodger et al., 1979; Alderink and Dietrich, 1983), and the total economic impact is conservatively estimated to be around $300 million/year in the United States (Okafor et al., 2019). While not all of regions of the United States have the same level of disease, the disease has been reported in nearly all of the 50 states.

Disease Impact on Industry

The impact of anaplasmosis is often referred to as a "significant hurdle to the profitability of cow herds in the United States" (Kocan et al., 2003; Kocan et al., 2010a; Reinbold et al., 2010c). However, the actual effect of the disease is not without contention and confusion. A 1976 survey in California estimated that anaplasmosis in cattle costs the livestock producers in that state approximately a 5.25 million dollars/year (Alderink and Dietrich, 1983). In 1982, Alderink and Dietrich attempted a robust accounting of the impact of anaplasmosis on the state of Texas and estimated a cost of $424 per clinical case and a total cost of anaplasmosis to the state's beef industry of $6.4 million/year. Both reports cited adult cattle mortality, reduced calf crop, increased cull rate in beef herds, veterinary fees and treatment costs as production parameters impacted by anaplasmosis. Morely and Hughes-Jones in 1989, estimated the cost of bovine anaplasmosis to beef producers in a region of Louisiana and subsequently extrapolated those costs to estimate a statewide cost of anaplasmosis of $1.02 million for the beef herds (Morely and Hugh-Jones, 1989a). Revised values by Alderink and Dietrich adjusted to a 2016 dollar value and updated morbidity and mortality increases due to anaplasmosis in cattle herds to cost to $627 per clinical case (Marsh, 2017). Other production losses in cattle associated with anaplasmosis include weight loss of both cow and calf, cull losses, abortion losses, milk production loss in dairy cows, delay in estrus in beef and dairy herds, decreased bull fertility,

loss of genetic potential of cows or bulls, loss of future production, decreased marketability in seedstock production, and decreased weight gain in orphaned calves.

Besides the fiscal and intrinsic losses experienced by afflicted herds, import and export of cattle is limited by anaplasmosis status. The U.S. State of Hawaii is an example of a state which requires cattle to be tested and confirmed negative before entry into the state (Importing cattle, 2019). Canada enforces a similar regulation for cattle entry to which they attribute the limited disease presence in the country (Howden et al., 2010). These protective burdens can limit the marketability of cattle from known infected areas, but may also limit producers in those protected regions in their genetic inputs and prove to be another obstacle to herd improvement. This phenomenon harkens back to the burden of the babesiosis in United States cow-herds following the Civil War (Strom, 2010), and is shared by cattle producing countries worldwide.

Transmission

Transmission to susceptible cattleoccurs through a variety of mechanical vectors (veterinary instruments and flies), biological vectors (ticks) (Kuttler and Simpson, 1978; Stewart, 1979; Eriks et al., 1989; Ewing et al., 1997; de la Fuente et al., 2003; Scoles et al., 2005; Lankester et al., 2006), and transplacental transmission (Kocan et al., 2010a; Kocan et al., 2010b; Aubry and Geale, 2011; Kocan et al., 2012). Nearly all methods of transmission appear to be dependent upon the strain of anaplasmosis as not all strains are infective for ticks or transmissible by flies (Kocan et al., 2004; Scoles et al., 2005; Kocan et al., 2010a; Kocan et al., 2012).

Veterinary instruments cane transfer infected blood to naïve cattle. Instruments such as injection needles, tattoo instruments, surgical instruments, and other tools canserve as a fomite for blood transfer from cattle infected with anaplasmosis to naïve cattle (Dikmans, 1950). Of

those mentioned, only injection needles have been published to transfer of blood products for the likelihood of disease transmission and cause anaplasmosis infection in cattle (Reinbold et al., 2010a). In their study, a needle was inserted into the muscle of a clinically infected animal and then immediately retracted and inserted into the muscle of a naïve animal. Of the study subjects, 6 of 10 naïve animals developed anaplasmosis following the treatment. Though differences from the field exist in the study, it is one of the best supporting documents for the efficacy of vectored transfer of bovine anaplasmosis using veterinary tools. To the author's knowledge, no studies have been conducted to understand the type of disinfection or time of contact required for the disinfection of surgical or tattoo instruments from anaplasmosis causing bacteria. Another veterinary tool which may have a role in transmission of anaplasmosis between cattle is rectal examination sleeves. While experimental demonstration of sleeve transmission exists for Bovine Leukosis Virus (Hopkins et al., 1988), none exists for bovine anaplasmosis.

Mechanical vectors also include flies which acquire blood meals fromtheir host and generally feed on multiple hosts before repletion. These flies include *Stomoxys calcitrans* and flies of the *Tabanid* genus. Scoles et al., 2005 was unable to demonstrate transmission of anaplasmosis to cattle from stable flies infected with Anaplamsa species. Previously however, Potgieter demonstrated transmission of anaplasmosis by stable flies, *Stomoxys calcitrans* to cattle (Potgieter et al., 1981), and Hawkins demonstrated transmission of anaplasmosis by four species of *Tabanus* to cattle (Hawkins et al., 1982). In review of the literature, the authors encouraged caution in the extrapolation of experimental transmission of anaplasmosis to cattle due to flies because there is a significant difference between experimental and field conditions with regards to the number of flies which may be present on cattle in the field and the limited numbers in studies have been performed to replicate consistent results (Aubry and Geale, 2011). For all

types of mechanical vectors to transfer anaplasmosis between cattle, infective ability appears to be dependent on the number of infective particle load transferred by the vector (Scoles et al., 2005).

Biological vectors are different from mechanical vectors because of their ability to be infected by a small number of bacteria which in turn are replicated to increase pathogen numbers in the mid-gut and salivary regions of the tick potentially culminating in 10^4 to 10^5 organisms per salivary gland at the time of transmission (Aubry and Geale, 2011). Not all ticks have the ability to be infected by *A. marginale* (Dikmans, 1950; Smith et al., 1986; Kocan 2004). An uninfected tick – generally a larvae, nymph or an adult male – can feed on an infected cow and become infected with an *Anaplasma* species, multiplying the bacteria in their mid-gut and then transmit anaplasmosis it to the next bovine the tick feeds on next. Larvae and nymph stage ticks can become infected with anaplasmosis and carry the infection into the next life stage – transtadial transmission (Kocan et al., 2010a). Adult male and female ticks can also become infected with anaplasmosis; but only adult male ticks are likely to feed on more than one host (cow). The female ticks cannot spread it to their offspring, referred to as transovarial transmission in ticks (Kocan et al., 2010a). Lack of transovarial transmission is a significant limitation for the maintenance of *A. marginale* in the tick population (Stitch et al., 1989; Kocan et al., 2003). The lack of long term infection also limits the ability for ticks to function as a reservoir for *A. marginale*. As such, naïve ticks must be infected as juveniles with anaplasmosis in order to infect cattle as adults. Maintenance of the anaplasmosis within the tick population is therefore dependent on the presence of infected cows as well as the ability of the tick vector to survive in favorable microhabitats in the environment.

Transplacental transmission of anaplasmosis in cattle has a significant role in the epidemiology of the disease within a cow-herd (Norton et al., 1983; Zaugg and Kuttler, 1984; Zaugg, 1985). One study demonstrated approximately a 15.6% of calves born to anaplasmsosis infected dams were positive for anaplasmosis at birth (Potgieter and Van Rensburg, 1987). Another report examined the effect of stage of gestation on transmission of anaplasmosis by inoculating naïve dams with blood from anaplasmosis positive cattle during each trimester of pregnancy (Zaugg, 1985). In this study, 1 of 2 inoculated cows in both the 2^{nd} and 3^{rd} trimester resulted in fetal blood that was infective for anaplasmosis to splenectomized calves (Zaugg, 1985). Interestingly, following parturition, while neonatal blood was positive for anaplasmosis antibodies, it was neither positive for inclusion bodies, nor infective to splenectomized calves (Zaugg, 1985). This report, while small (n=6), demonstrates the variability in vertical transmission of anaplasmosis in host cattle and introduces a possible role of the developing fetal immune system limiting transmission of anaplasmosis to neonates (Zaugg, 1985). Similar to other mechanisms of transmission of anaplasmosis, these dynamics are possibly related to the *Anaplasma* species or *Anaplasma* strain diversity which exists within a cattle herd.

Pathology and clinical signs

Anaplasma. marginale gains entry to the host vascular system transdermally or through exchange of blood or bacterial products where it can access host erythrocytes, the primary site of *A. marginale* replication in cattle. Once replication occurs inside the host erythrocyte, the bacterium forms inclusion bodies. These inclusion bodies then rupture and release rickettsial packets which parasitize new erythrocytes. During acute anaplasmosis infection, the population of host erythrocytes are heavily parasitized and the percent of parasitized erythrocytes (PPE) can range from 30 to 60% potentially reaching 10^9infected erythrocytes per milliliter of blood

(Palmer et al., 2004). The prepatent period of anaplasmosis infection varies in cattle due to infective pathogen dose. The prepatent period of anaplasmosis in cattle ranges from seven to 60 days, with an average prepatent period being approximately twenty-eight days (Palmer et al., 1999; Kocan et al., 2003; Kocan et al., 2012). High percentages of cattle erythrocytes become infected during acute anaplasmosis infection, and removal of damaged erythrocytes by the splenic reticuloendothelial system correlates with the severity of anemia and icterus (Richey, 1981; Kocan et al., 2003; Kocan et al., 2012; Kocan et al., 2015). Mortality rates of newly infected cattle is reported between individuals 30-50% (Richey, 1991; Kocan et al., 2003). Individuals that survive anaplasmosis infection may recover, but are persistently infected and serve as a reservoir for transmission of anaplasmosis to naïve individuals (Kocan et al., 2003; Kocan et al., 2010b; Aubry and Geale, 2011).

Clinical diagnosis of bovine anaplasmosis is commonly made on history and presentation of clinical signs in the animal. Symptoms of clinical anaplasmosis infection in cattle include fever, jaundice, aggressive behavior, abortion, respiratory distress, depression, lethargy, anorexia, and sudden death (Howden et al., 2010; Kocan et al., 2010a; Hairgrove et al., 2014). A clinical case of anaplasmosis in cattle may include one or all of these signs and can range from mild to severe, with recovery periods ranging from several days to several months depending on severity, age, and stage of production (Palmer et al., 1999; Kocan et al., 2003; Kocan et al., 2012). Clinical signs of anaplasmosis more commonly appear in older animals, greater than two years of age, while younger animals are less likely to present with severe clinical signs (Kocan et al., 2010a). Anecdotal reports of clinical anaplasmosis cases, and even deaths, exist in cattle as young as 18 months during periods of increased stress or concomitant disease pressure.

The immunological host response to *A. marginale* infection is determined by the host evasion mechanisms possessed by the bacterium, namely the mutation of surface antigens which are recognized by the host immune system (Knowles et al., 1996; Brayton et al., 2002; Palmer et al., 2006). The development of new antigenic variants allows for evasion of the host antibody presence and subsequent increase in *Anaplasma* bacteria resulting in newly infected erythrocytes (French et al., 1998; French et al., 1999). As previously stated, acute anaplasmosis infection may result in levels of parasitized erythrocytes nearing 10^9 per milliliter of blood. Once the antigenic *Anaplasma* variant is recognized and targeted by the host, bacterial growth is stopped and returned to a level ranging between 10^2 and 10^6 infected erythrocytes per milliliter of blood – below the microscopic detection threshold of $10^{7.2}$ (Eriks et al., 1993). While different patterns have been described, this repetitive rise and fall of *Anaplasma* bacteria and host antibody production characterizes the lifetime of the infection in persistently infected individuals (Kieser et al., 1990; French et al., 1998; French et al., 1999; Palmer et al., 1999). The cyclic pattern also has direct effects on the ability of diagnosis of anaplasmosis in cattle as the detectable bodies of inclusion bodies, antibodies, and rRNA are subject to the repetitive fluctuation.

Laboratory diagnosis of bovine anaplasmosis is conducted for disease confirmation in an individual animal, epidemiological disease classification in a cattle population or for genomic characterization of the *Anaplasma* bacterium including strain differentiation or antibody differentiation. In the past, several methods of testing for the presence of anaplasmosis antibodies included complement fixation (CF) tests, rapid card agglutination (RCA) tests, and blood smears. These test methods were demonstrated to possess severe limitations of sensitivity and/or specificity and potentially resulted in the misclassification of test subjects (Coetzee et al., 2007). The gold standard for diagnosis of anaplasmosis infection is the injection of potential

anaplasmosis infected blood into a splenectomized sentinel bovine (Reinbold et al., 2010a), . Cattle with no spleen do not have a reticuloendothelial system therefore erythrocytes in the sentinel bovine would be parasitized by anaplasmosis and the animal would demonstrate clinical signs of anaplasmosis. Subsequently, blood smears from these sentinel cattle would reveal tell-tale inclusion bodies on the margins of the erythrocytes associated with anaplasmosis infections. Currently, it is common for researchers and diagnosticians utilize competitive enzyme-linked immunosorbent assay (cELISA) and reverse transcription polymerase chain reaction (RT-PCR) tests to diagnosis anaplasmosis in cattle. The current anaplasmosis cELISA test utilizes the conserved major surface protein 5 (MSP5) which is present across all known strains of *A. marginale*. The anaplasmosis PCR diagnostic test utilizes the rRNA for the 16S subunit and the MSP5 of the bacterial genome. The reported diagnostic sensitivity and specificity for the anaplasmosis cELISA test is 99.9% and 99.7% (Chung et al., 2013), respectively while the anaplasmsosis RT-PCR tool is reported to be 99.9% and 99.9%, respectively (Reinbold et al., 2010a; Reinbold et al., 2010b).

Distribution

Bovine anaplasmosis has been reported to be the most significant tick-borne pathogen of cattle across the world (Kocan et al., 2003; Kocan et al., 2010a). In the United States, anaplasmosis has been reported in cattle located in all 48 contiguous states (Kocan et al., 2003; Kocan et al., 2010a). In the Midwestern United States, the American Dog Tick (*Dermacentor variabilis*) and the Moose tick or Winter tick (*Dermacentor albipictus*) are ticks that are able to be infected with anaplasmosis. In the West and Northwest US, the Rocky Mountain Tick (*Dermacentor andersoni*) and the Pacific Coast Tick (*Dermacentor occidentalis*) are known known biological vectors for anaplasmosis (Scoles et al., 2005). As the worldwide distribution of

anaplasmosis does not match entirely the distribution of known vectors as researchers suspect that more tick species of havethe ability to be infected with anaplasmosis (de la Fuente et al., 2004; Kocan et al., 2010a). The distribution of anaplasmosis cases in cattle is commonly considered to be associated in herds located in the southeastern United States, however, anaplasmosi has a considerable presence and poses a serious risk to cattle production in the northwestern United States and throughout the Midwestern states (Safford, 1965; Utterback et al., 1972; Zaugg and Kuttler, 1985; Hairgrove et al., 2014; Hairgrove et al., 2015). Spread and maintenance of the anaplasmosis within novel environments is dependent on the distribution and movement of infected cattle as well as the ability of the tick vector to survive in favorable microhabitats (Kocan et al., 2010a; Kocan et al., 2010b; Aubry and Geale, 2011). Tick microhabitats may be present in environments with climate patterns that seem unlikely to support ticks such as cooler or drier climates found in the northern and western United States and Canada.

Many studies have investigated and reported the regional prevalence of *A. marginale* in U.S. states such as Texas, Louisiana, California, Idaho, and Georgia, but few randomized studies have been attempted to assess statewide prevalence (Utterback et al., 1972; Alderink and Dietrich, 1983; Zaugg and Kuttler, 1985; Morely and Hugh-Jones, 1989b; Hairgrove et al., 2014; Hairgrove et al., 2015; Okafor et al., 2019). Spatial modeling based on diagnostic laboratory submissions has also been used to describe the likely spread and distribution of the anaplasmosis in cattle across the state of Kansas (Hanzlicek et al., 2016).

From an epidemiological standpoint, confusion exists regarding the difference between the anaplasmosis prevalence within a herd and the anaplasmosis prevalence across herds in a specific region. The within herd prevalence describes the number of animals within a given

population (herd) who are infected with anaplasmosis as a proportion of the number of animals in the entire population. In this manner the within herd population is described as a percentage anaplasmosis infected of the whole. Few studies have undertaken the characterization of multiple herds and their respective within herd prevalence of anaplasmosis infections. A simple across-herd prevalence could be arrived at by describing the anaplasmosis infection status of individual herds, either positive or negative, using samples from a pre-determined number of individual cattle within those herds. This type of prevalence can be used to describe the proportion of herds in a region which have the disease present often expressed as a percentage of anaplasmosis infected herds.

Few studies have undertaken the necessary sampling to characterize the prevalence of multiple herds of differing anaplasmosis prevalence to examine the effects of different levels of anaplasmosis infection on production or transmission within the herd. Therefore dynamics of within-herd transmission of anaplasmosis is relatively unknown in cattle (Kocan et al., 2010b). Since anaplasmosis must be present in the host animal before the vectors found in the environment can pose a threat, the higher the within-herd prevalence could be associated with greater risk for naïve animals within the herd to become infected with anaplasmosis. Conversely, the lower the within-herd prevalence with anaplasmosis, the lower the risk there is for naïve animals becoming infected. These postulates, while based in the epidemiology of disease pressure are assumed and may not effectively describe the effects of the disease in the field.

Across-herd prevalence of anaplasmosis may impact disease transmission directly, but it is a characterization which must be carefully interpreted before application. This cautious interpretation is due because an across-herd prevalence of anaplasmosis in cattle consists of descriptions of herds which have individual cattle within-herd prevalence measures of their own.

For example, a 100% across-herd prevalence of anaplasmosis may describe herds within a region that each have less than 1% of the population infected. The risk that cattle in herds with low anaplasmosis prevalence pose in regions with high across-herd prevalence pose to other cattle populations is unknown at this time compared to the risk of infection from herds with high within herd prevalence in a region with low across-herd prevalence.

Treatment and Prevention

Anaplasmosis is clinically described as both the herd and individual level cases. Therefore, treatment for anaplasmosis must be defined as treatment of individuals for acute infection, treatment of individuals for chronic infections, treatment of herds for acute infections, treatment of herds for chronic infections. Treatment of acute anaplasmosis infections in individual cattle involves restraint and delivery of parenteral long-acting tetracycline, or the conditionally licensed fluoroquinolone, enroflaxacin. Though these medications have been shown to be experimentally effective at reducing parasitemia in the anaplasmosis infected animal, the process of treatment can be enough stress to lead to the demise of the infected animal. As described in the pathology of the disease, even at the onset of clinical signs, the anaplasmosis infection will have been active for approximately 14-21 days and the level of parasitemia and anemia can be extreme enough to make treatment at that time a risk for death (Kocan et al., 2012). Therefore, individual treatment is most beneficial when accomplished at the earliest sign of clinical disease in anaplasmosis cases.

With the relevant questions around the impacts of the disease, livestock producers and veterinarians are faced with several options regarding the management of anaplasmosis within individual herds. These options are to use 1) intervention using diagnostic testing and re-

allocation of cattle, 2) anaplasmosis vaccination, 3) antibiotic therapy, 4) vector control, or a combination of multiple methods.

1) Testing and Culling

As with other diseases in which widespread diagnostic testing has utility, animal management following anaplasmosis testing is a consideration of importance. The economic outcomes of a test and cull strategy verses an isolation strategy should be weighed against each other in controlling anaplasmosis in cattle. Also, the impact of anaplasmosis infected animals leaving one herd and going to another must be considered. Testing and culling for anaplasmosis also carries a significant capital investment as each animal should be tested to ensure the thoroughness of the distinction between infected and naïve animals within the group. Testing and culling or isolation cattle with anaplasmosis is also challenged with consideration of biological vectors in the environment may harbor the bacteria for the duration of their lifetime and may propagate the infection into naïve animals who are re-introduced following testing. Therefore, testing and culling or isolation for anaplasmosis should be carried out over multiple years following the vector season in the given environment to allow for the new infections which arise due the presence of infected arthropods.

2) Vaccination

The current approved anaplasmosis vaccine in the United States is domestically produced and is a killed vaccine manufactured from purified infected red blood cells. It was previously commercially licensed and marketed, but is now conditionally licensed by the United States Department of Agriculture for use in multiple states (Hart et al., 1990). The vaccine is marketed for the reduction of severity of clinical signs of anaplasmosis cases within the immunized animal, however, animals which are immunized may still become infected with the disease and remain infected carriers for life. Questions regarding the cross-strain anaplasmosis protection of cattle with the vaccine exist, but are not answered in the current literature. One important impact of anaplasmosis vaccination its effect on the utility of the cELISA diagnostic test in the herd of interest as the antibody production of the vaccinated animals is indistinguishable from the antibody produced naturally upon infection. Therefore, the PCR test becomes the diagnostic test of choice for both screening and confirmation of anaplasmosis in the vaccinated herd.

3) Antibiotic therapy

Antibiotic therapy has been used to treat both clinical and chronic infections of cattle with bovine anaplasmosis. Literature supporting the use of oxytetracycline in both clinical and chronic anaplasmosis cases has been the mainstay of treatment (Magonigle and Newby, 1983; Corely L, 1984; Richey EJ, 1992; Kocan et al., 2010; Bovine anaplasmosis, 2015). Recently, the fluorquinolone enroflaxacin has been approved as a potential option for the treatment of acute infections in bovines. Due to the severity of clinical signs and the challenge of treatment with appropriate dose without added stress in an acute infection, treatment success of acute anaplasmosis infections are mixed in cattle.

Oral tetracycline has long been a mainstay of anaplasmosis infection control, but in 2017, the Veterinary Feed Directive was implemented by the United States Food and Drug Administration limiting the use of in-feed antibiotics to label indications and dosages (FDA, 2015). In the context of the management of bovine anaplasmosis, this ruling placed a restriction on commonly used extra-label dosages of chlortetracycline which were delivered in mineral formulations, fed in supplement form, or mixed into rations for the management of anaplasmosis within a population. The feeding of chlortetracycline is still allowable under the VFD, but the current literature is unclear regarding the efficacy of the dosage and the expected outcomes for its delivery when fed according to label directions. Current label directions for in-feed chlortetracycline pertaining to anaplasmosis 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 weight/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 (CFR, 2020).

Debate over the utility of chlortetracycline for the management of anaplasmosis centers around the likelihood of chemosterilization in persistently infected cattle and the efficacy of the drug in controlling or preventing infections in naïve animals. Chemosterilization of anaplasmosis antibody in cattle itself is somewhat of an enigmatic principle in the field, in that chemosterilization of infected cattle can be diagnostically confirmed only to the length of time which is allowed to pass before a confirmatory test may be performed during which the animal may be re-infected due to vector exposure or relapse due to incomplete clearance initially. The length of time may be extended, but until observed in a vector-free environment, chemosterilization is limited to the extent of time before which a confirmatory test is

administered. Multiple studies have demonstrated that cattle infected with anaplasmosis which have been chemosterilized are susceptible to reinfection with anaplasmosis (Richey et al., 1977; Reinbold et al., 2010b; Kocan et al., 2012). Therefore, the utility of chemosterilization of cattle infected with anaplasmosis is thus far disputed in large-scale commercial settings.

Chlortetracycline is labeled to control anaplasmosis in beef cattle herds by: 1) In the case of a naïve animal consuming an appropriate amount of chlortetracycline which is exposed and does not develop infection nor antibodies indicating infection, 2) in the case of a naïve animal which is exposed and prior to the development of clinical signs, receives chlortetracycline at an appropriate dose and does not develop infection nor antibodies indicating infection, or 3) in the case of naïve animals housed with infected animals who, because of consumption of appropriately dosed chlortetracycline lack the transmissive capacity to cause a new infection. These three forms of controlling possible anaplasmosis outbreaks are supported in the literature through the appropriate timing and dose delivery of in-feed chlortetracycline (Brock et al., 1959; Franklin et al., 1965; Richey et al., 1977; Reinbold et al., 2010b). Therefore, the accurate delivery of chlortetracycline has potential to function as an important tool for cattle producers and veterinarians to control anaplasmosis in beef herds.

4) Vector control

The application of an external parasite control program for the control of vector activity in the environment is another method for the management of bovine anaplasmosis in a cow herd. Strategies for controlling external parasites include chemical, range management, and manure management. Chemical management of ticks and flies in cattle production includes topical parasiticides, parasiticide impregnated ear tags, and feed through insecticides. Range management of cattle vectors includes prescribed burning and patch burning (Polito et al., 2013). Manure management of cattle vectors such as ticks and flies includes rotational grazing and intensive stocking management. Current evidence presents challenges for each method of vector control as the impact of mechanical and biological vectors are dependent on the strain of *A. marginale* (Smith et al., 1986; Kocan et al., 2003; Kocan et al., 2010a). Regions in which competent tick vectors are not present may depend on unknown tick vectors or the presence of mechanical vectors such as flies which are competent to spread the disease (Kocan et al., 2003). In the absence of a complete understanding of the vectors and their impact on transmission of anaplasmosis to cattle, livestock producers in all regions must account for the potential impact of all potential vectors to control the disesase. Few studies have been undertaken to measure the impact of these common interventions to control possible insect vectors on the presence of anaplasmosis in cattle herds and subsequent impact on regional livestock production.

Conclusion

Bovine anaplasmosis has been described to be a significant hurdle for profitability in both the global and United States production of cattle. The ability of the disease to evade host immune defenses and its diversity of vector transmission has presented a challenge for United States beef cattle production throughout history. As with other diseases, no silver bullet exists for the

management of anaplasmosis in cow herds across the United States. Instead, the challenges controlling anaplasmosis in cattle of each geographical region require producers and veterinarians to consider the available prevention methods based on the ability for beef cattle operations to carry out a practice. The current literature supplies some of the answers to prevention, control and treatment of anaplasmosis in cattle but the same publications allow one to ponder new questions and new directions in the face of new legislation and novel control measures for anaplasmosis. Therefore the objectives of this dissertation are to examine and describe current vector epidemiology of bovine anaplasmosis as it relates to hematophagus arthropods, the geographical/distributive epidemiology of bovine anaplasmosis in the host animal in the state of Kansas, and the management of anaplasmosis in naturally infected cattle using currently approved antibiotic therapies in a commercial setting.

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Chapter 2 - Diversity and Phenology of Tick Species in the Flint Hills Ecoregion (United States)

Abstract

Bovine anaplasmosis is a tick-mediated disease of cattle which presents a significant hurdle to livestock production across the world. The rickettsial agent, *Anaplasma marginale*, is known to infect multiple species of ticks who then act as biological magnifiers in the environment. In the Flint Hills ecoregion (USA), *Dermacentor variabilis* is the known vector, while other ticks are unable to vector the disease. The objective of the present study was to evaluate the density and phenology of questing ticks collected in the Flint Hills. In total, 10,557 questing ticks were collected during a two-year study period from 2015–2017 from sites in the Flint Hills. In both years, three Ixodid species were collected with adult emergence differences amongst the species, and consistent emergence of *D. variabilis* in late July. Statistical modeling was used to evaluate relationships between tick density and climatological variables. Accumulated temperature and photoperiod are significantly associated with all three tick species collected in the study. The normalized difference vegetative index was negatively associated with the density of *Amblyomma americanum* ticks(p=0.017) while saturation deficit was significantly but negatively associated with *D. variabilis*($p=0.038$) and maximum relative humidity was negatively associated with *Amblyomma maculatum* ticks($p=0.041$). The increase in adult density of *D. variabilis* in late July will be a helpful guide for understanding regional and within-herd transmission dynamics of bovine anaplasmosis. Further research is warranted in the year-to-year variation in the emergence of the tick as well as the ability of the ticks within a region to harbor the disease without continued reinfection from reservoir cattle.

Introduction

Ticks are economically important pests that affect livestock production worldwide due to blood meal demands and conference of significant diseases. Estimated losses due to bovine anaplasmosis, a tick-mediated disease of cattle ranges in the several millions of dollars every year in the US alone. For instance, older estimates of the cost of a clinical bovine case due to anaplasmosis was estimated to be around $400/animal (Goodger et al., 1979; Alderink and Dietrich, 1983), and the total economic impact is conservatively estimated to be around $300 million/year in the US (Okafor et al., 2019). Losses due to other tick-borne diseases to livestock and companion animals are not readily available but can be reliably expected to be in several hundreds of millions each year. Tick-borne diseases to humans and animals in the midwestern US have increased in their frequency and intensity over the recent years (e.g., Raghavan et al., 2014; 2016), particularly the spatiotemporal pattern of bovine anaplasmosis in the midwestern US has steadily worsened in the past decade (Hanzlicek et al., 2016).

Recognized as one of the world's last remaining Tallgrass Prairies, the Flint Hills ecoregion in the Midwestern US represents an area of great significance for livestock production in the US. Poorly suited for cultivation and crop production, the area is recognized for its superior grass quality and resultant grazing performance by ruminant species of which cattle dominate. Due to the seasonality of the grass quality, many million cattle transition to pastures in the Flint Hills from other parts of the US as young growing cattle (i.e. stockers) and as transitory reproductive females (i.e. cow herds), adding origin diversity to the complexity of the grazing population.

The current study evaluates the diversity of different questing tick species and their phenology in Flint Hills ecoregion by conducting tick surveys at 12 locations over a two-year

period between 2015 – 2017. The information generated in this study is essential to understand the reasons behind current increase in bovine anaplasmosis cases in the region and for improving the management of this economically important disease. Additionally, the data generated in this study will serve as a baseline for tick diversity and phenology for understanding the dynamics of tick populations in this ecoregion due to ongoing climate change and ecological forces.

Materials and Methods

Study area

This study was conducted in the Flint Hills, a narrow north-to-south region located in eastern Kansas and northcentral Oklahoma, designated by the US Environmental Protection Agency as an ecoregion characterized by dense coverage of different tallgrass species. Flint Hills ecoregion covers an area of 25,333 km^2 with wide interannually varying continental climate. Climate conditions here range from extreme heat in summer with highs ranging between 26-43° C, and lows in the winter dropping to -29° C. Precipitation varies highly from year to year with average precipitation received in the area being approximately 84 cm. Flint Hills landscape is mainly composed of prairie vegetation dominated by big and little bluestem, switch grass, and Indian grass. Trees are seldom found, except along stream and river bottoms where they are abundant, creating significant areas of forest-field interface.

Tick survey and classification

For the evaluation of tick diversity, questing ticks were collected from twelve locations between the months of March and August using a 1 m^2 white flannel cloth attached to a 1.3 m long flagpole for a fixed amount of time (1h). Ticks attached to the flannel were collected using forceps at every 2 – 5 m distance and stored in a plastic container until they were brought to the

laboratory, placed on dry ice. Tick surveys were performed by four similarly trained biologists during the entire study period, but tick collectors changed once in the second year of the study.

To study the phenology of ticks, three sites were selected, in the north, central, and southern Flint Hills ecoregion to represent any potential climate variation. From these sites, ticks were collected from 300 m^2 transects, once a month from the beginning of March 2015 through end of March 2017, as described above, except for periods when the transects were under snow cover and/or temperature was below freezing. No time limit was set and entire transects were swept for ticks over the vegetation cover. Once in the lab, ticks were placed in -20° C for 48-56 hours after which time they were thawed and microscopically identified to their species level using N. American tick taxonomic keys (Coley, 2015). Nymphs and larvae were first identified using morphological keys, and to further confirm the species, a subset of these ticks was evaluated using molecular analysis using PCR technique.

Sample handling and DNA extraction

Ticks were first thawed to room temperature and pools of 10 larvae per pool and 10 nymphs per pool were individually prepared. Total genomic DNA from these pools were extracted using the QIAGEN DNeasy Blood & Tissue kit (QIAGEN, Valencia, CA) following the recommended manufacturer's protocol with the following exceptions. The tubes were homogenized in 2ml Lysing Matrix M Screwed caped tubes filled with ¼" (6.35mm) diameter of Zirconium Oxide ceramic grinding sphere (MP biomedicals. LLC), and 180 µL of ATL buffer (QIAGEN, Valencia, CA), 25 µL of proteinase K (QAIGEN, Valencia, CA). The tubes are incubated at 56°C with shaking for an hour in a Disruptor Genie cell disruptor (Scientific Industries, Inc, NY), and followed the kit instructions. DNA concentration was measured by

Nano drop spectrophotometer (ThermoFisher, Wilmington, DE) and stored at -20°C for PCR detection of gDNA.

Positive control construction

Positive amplification controls including those for *A. americanum, A. maculatum, and D. variabilis* were synthesized and cloned into a plasmid vector and used as controls.

Real-time PCR protocols

Oligonucleotides used in this PCR reactions were listed in Table 1. The singular real-time PCR reactions were conducted with Bio-Rad CFX96 Real-time PCR detection system with the iQ Multiplex Powermix kit (BioRad, Hercules,CA, USA). For each PCR reaction, a 20 µL reaction was prepared, which contained 10 µL 2X IQ Powermix, 5 µL DNA template, 1µL probe, 2 µL primer mix, and 2 µL nuclease free water. An initial denaturation at 95^0 c for 10 minutes was followed by 45 cycles of 95^0 c for 15 sec, the annealing and extension temperature at 65^0c for 45 seconds. The end results are analyzed using Bio-Rad CFX Manager 3.0 software. Annealing temperatures for individual PCR assays were first optimized using temperature gradient PCR (Bio-Rad CFX96). The optimal annealing temperatures of the assay were determined based on signal intensities of the PCR amplifications. Ct value $\leq$ 30 are strong positive reactions obtained by abundant target nucleic acids in the samples. Amplification products are analyzed by electrophoresis by 1.5% agarose. The expected amplicon sizes for the respective tick species are mentioned in Table 1.

Environmental data

Daily climate data for the study period, closest to the sampling locations were obtained from the National Weather Service (NWS) and cooperative stations. This data included temperature (minimum, maximum, and mean), relative humidity, precipitation, and wind speed. Daily evapotranspiration and soil moisture index were derived from the raw climate records. Vegetation index data for this analysis was extracted from the MOD13A2 product derived from the MODIS instrument onboard the EOS-Terra platform. Vegetation indices from this product (NDVI, EVI) are calculated from the blue, red, and near infrared MODIS bands using daily atmosphere-corrected bidirectional reflectance values over 16-day compositing periods. Composite index values for the 16-day periods are selected to represent each compositing period by culling all pixels whose quality assurance metrics fall below a minimum standard, then selecting the highest index value from the view angle that is closest to nadir. The study site was located on the H10V05 MODIS tile.

Statistical analysis

The association between climate and land cover covariates with tick density for each species was evaluated in a Generalized Linear Mixed Model construct using the glmm package (see Knudson, 2018) in R-Statistical program (R Core Team, 2019). The correlation among covariates were first assessed and pairs of covariates with R value >8 where Model over-fitting can lead to finding erroneous associations. Therefore, before fitting the glmm model, the strength of associations between tick density and different fixed-effect covariates were individually screened by fitting univariate models with a liberal cut-off *P*-value. Only those fixed-effect covariates that retained statistical significance ($P \leq 0.1$) were kept for the glmm model; in which, log normalized tick density was the dependent variable, different climate and land cover

covariates were fixed-effect independent variables, and location, and month and year of tick collection were kept as random-effect variables. A full model with all screened variables and random effects was fitted first, followed by several mixed models with fewer fixed-effect covariates at each step without those that were non-significant ($P \leq 0.05$). These steps were followed independently for each tick species.

Results

During the two-year study period from 2015 – 2017, a total of 10,557 ticks were collected from 12 collection sites in the Flint Hills ecoregion; 8,083 (76.5%) of these ticks were identified as *A. americanum*; 1,481 (14.0%) as *D. variabilis*; 965 (9.14%) as *A. maculatum*; and, 28 (0.26%) were *Ixodes scapularis*. The male/female ratio for all four species did not change during in the two years that ticks were collected, and in general, there were more females relative to males for all species in both years. The sex ratio for *A. americanum* was 0.61:1 (male: female), for *D. variabilis*, 0.69:1, and for *A. maculatum*, 0.78:1. The sex ratio for *I. scapularis* could not be reliably estimated due to the low number of samples collected. All life-stages of *A. americanum* and *D. variabilis* were collected from all sampling sites, whereas *A. maculatum* was not collected from study sites located above. *Ixodes scapularis* was collected only from two study sites in the southern part of the study region.

Among *A. americanum* ticks, larvae (33.13%) and nymphs (33.55%) contributed to a large portion of the collection, followed by 20.64% females and 12.66% males. Larva and nymphs of *D. variabilis* and *A. maculatum* were not collected by flagging; the proportion of female and male ticks of these species were (41%, 59%) and (44%, 56%), respectively.

A total of 4,557 ticks were collected from the three long-term sites that were sampled each month throughout the study period to evaluate the phenology of ticks. From these sites, *A.*

americanum male (n = 319), female (n = 523), nymph (n = 807), and larva (n = 969); and adults *of D. variabilis* (n= 466; male = 191, female = 275) and *A. maculatum* (n = 163; male = 63, female = 100) were collected (Table 2). All species of ticks were present in the three long-term sites. Regardless of the collection site, the three major tick species found in the Flint Hills ecoregion displayed a unimodal density distribution in both years (Figure 2.1, 2.2, 2.3). Noticeable overlap in the emergence of the three post-emergent life-stages of *A. americanum* ticks is evident but the nymphs emerged relatively earlier in the season followed by adults and larvae. In both years, the peak activity of *A. americanum* nymphs occurred in May, adults peaked in June and then larvae in August. Adult density of *D. variabilis* increased in July but the emergence of adults of *A. maculatum* occurred earliest in the year among all three ticks, in May.

The final models for each species that evaluated climate associations with tick density revealed that accumulated temperature and photoperiod are significantly associated with all three tick species collected during 2015 – 2017 in the Flint Hills ecoregion (Tables 3 – 5). The NDVI was associated with the density of *A. americanum* ticks while saturation deficit, a measure of drying power of the air was significantly but negatively associated with *D. variabilis* (Table 4), and maximum relative humidity was negatively associated with *A. maculatum* ticks (Table 5). The random effect parameters, month of collection and location was significant in all glmm models.

Discussion

The objective of this study was to determine the diversity and phenology of questing ticks in an area where livestock production is a major economic driver. To the collective knowledge of the authors, intensive tick surveys, as done in the present study, investigating the diversity and/or phenology of ticks has not been conducted before for this region in recent decades. The findings

in this study therefore provide new insight into the tick ecology in this livestock intensive region, which will be useful for future studies and for investigating and designing management practices to limit tick density.

One such management practice could be the potential adjustment of the timing or method of burning seasonal grasses, a practice that is routinely followed by cattle ranchers in the region. Patch burning has been examined as a possible method for the reduction of tick burden in areas of intensive grazing (Polito et al, 2013). This study reported no difference in questing ticks between burned and unburned pastures, however, the researchers described a significant reduction of ticks found on cattle in pastures which were prescribed patch burning. The utilization of prescribed burning bears continued investigation in the reduction of leaf litter and the effect on tick abundance and behavior. A study in Missouri reported more adult *D. variabilis* ticks captured in the open field ecosystem than in the forest ecosystems sampled (Petry et al., 2010). This phenomenon was not observed or measured in the current study, but may affirm the importance of open grazable acreage in livestock systems and the ability to influence those ecosystems for the reduction in tick burden.

The distinctive and consistent unimodal phenology of questing adults of all three tick species and the immature stages of *A. americanum*, for both years indicate that there is currently only a single season during which these ticks are active in the Flint Hills. Too few *I. scapularis* ticks were collected in this study to make robust conclusions, but the adults of this species were all collected during late fall, indicating a cool-season peak for the adults and potentially its immature life-stages. Previous studies of *I. scapularis* ticks from the region indicate a similar activity period (White and Mock, 1991; Kollars et al., 1999). Tick density for the other three species appears to increase as temperatures warm up in early spring and last until early Fall;

however, the peak activity for these species (and for different life stages of *A. americanum*) occur at different times during this roughly 6-month period, often within a narrow one or two month time-frame. These results agree with reported results from Polito's description of the peak tick activity observed during the investigation of the effects of patch burning (Polito et al, 2013).

Accumulated temperature and photoperiod are reliable predictors of tick density in the Flint Hills, a finding similar to that reported in a recent study by Remesar et al., 2019 on *Ixodes ricinus* ticks in Spain (Remesar et al., 2019). Ticks are poikilothermic arthropods whose survival and fitness are closely linked to ambient conditions. Temperature and moisture available in the atmosphere, represented either as relative humidity or saturation deficit, have been long shown to exert significant effect on the questing behavior (e.g., Schulze et al., 2001; Harlan et al., 1990), development rate (Sonenshine 1985; Haile et al., 1987; Yoder et al., 2012), and reproduction and survival (e.g., Campbell et al., 1979; Civetello et al., 2008) of different *Ixodid* ticks, and they are a major limiting factor of the geographic distribution of a tick species (e.g., Raghavan et al., 2019). NDVI, an index for remotely detecting photosynthetic activity of plants is associated with *A. americanum* density in the present study. NDVI has been shown to correlate with the life history traits of different ticks and has been widely discussed as a reliable predictor of tick phenology in many cases (Randolph 2000). The phenology of other ticks found in Flint Hills appears not to be predictable using NDVI, as is the case with some other *Ixodid* tick species (Remesar et al., 2019).

The density of adult *D. variabilis* ticks increased as the saturation deficit increased, indicating that the emergence and questing behavior of the adults of these ticks coincided with drier conditions in the Flint Hills. However, it is important to consider the effect of saturation deficit not in isolation but in the presence of accumulated temperature and photoperiod as these

covariates together contributed to the mixed model (Table 3). The effect of photoperiod on *D. variabilis* has been shown to affect the questing behavior (Smith et al., 1941; Yoder et al., 2016), with longer photoperiods increasing questing activity. Similarly, the maximum relative humidity recorded per month is associated with the density of *A. maculatum* ticks, with lower relative humidity favoring increased density.

Juvenile (larvae or nymph) life stages of *D. variabilis* were not identified among the collection of ticks in this study. These life stages were reported in a sample collection of over 10,000 individual ticks in Missouri (Petry et al, 2010) and were found to be more commonly collected in forested areas while questing adult *D. variabilis* were more commonly collected in drier grassy areas. While the juvenile life stages of *D. variabilis* preferentially feed on small mammals, they have been shown experimentally to feed on bovines as juveniles (Stitch et al, 1989). Therefore, though larval activity of *D. variabilis* was not described in the current study, it has been reported previously in the southeastern United States and may remain a significant phenomenon in the impact of the tick as a biological magnifier for multiple pathogens (Kocan et al, 1980).

All questing tick specie collected in this study have been implicated in the transmission of pathogenic agents to humans and animals and are therefore intensely researched. Apart from the well-known pathogens transmitted by *A. americanum* (see: Childs and Paddock, 2003), the nymphs of these ticks were recently confirmed as transmitting agents of Heartland virus (HRTV) (Savage et al., 2013), and Bourbon virus (BRBV) (Savage et al., 2018), which were responsible for human deaths, proximate to the study area in the states of Missouri and Kansas (USA). The intensity and frequency of other diseases transmitted by *A. americanum* have been steadily worsening over the recent years (Raghavan et al., 2016a; Raghavan et al., 2016b), and recently it

was shown via ecological niche modeling that the potential geographic distribution of this species is broader in N. America than what was previously thought (Raghavan et al., 2019).

Transmission of *Anaplasma marginale*, the rickettsial causal agent of bovine anaplasmosis occurs via tick bites as well as mechanical means. Scoles et al described the transmission efficiency of competent tick vectors as 300 times as efficient than the mechanical transmission of biting flies (Scoles et al, 2005). Infected *D. variabilis* ticks maintain and biomagnify the disease in the environment and they are therefore an important component of the epidemiology of this disease and a target for management. The incidence of bovine anaplasmosis cases among cattle herds in the Flint Hills is not publicly available but two previous studies (Spare et al, 2020; Hanzlicek et al., 2016) indicate that many counties in this region are high-risk area for this disease. The role of ticks in the transmission of bovine anaplasmosis in the region is well accepted, however, their impact on within-herd and across-herd transmission on an annual basis is poorly understood. Specifically *D. variabilis* ticks spend 94-97% of their lifetime off the host animal, a Canadian study reported the competency of wild caught *Dermacentor* ticks to transmit the bacterium (Lankester et al., 2006). Elsewhere, *D. albipictus*, *D. andersoni*, and *D. occidentilis* have been implicated in transmission (Zaugg et al., 1986; Ewing et al., 1997; Aubry and Geale, 2011; Tucker III, et al., 2016). These reports highlight the importance of understanding the microenvironment and locomotive mechanisms which effect the distribution of competent vectors for management decisions to influence the transmission of anaplasmosis within a herd. The current study reported the capture of only one *Dermacentor* species in the collection area, and as a three-host tick, further research is warranted on *D. variabilis'* role in the maintenance and transmission of bovine anaplasmosis in the region.

In a study in Missouri, the researchers indicated a significant effect of collection method and collection environment on the species and life stages that are represented in a collection (Petry et al, 2010). The current study collected questing ticks by the method of dragging areas for ticks. As a result of the different questing behaviors of the various tick life stages as well as the preferred method of collection, the samples obtained may not represent the actual tick populations present at the collection locations. However, the surveillance of the land area as an ecoregion as opposed to another politically determined boundaries may have provided important reduction in heterogeneity in the environment inhabiting tick species. The prevalence of different pathogens among ticks in Flint Hills was not available at the time of this report but such data will shed more light on the nature of risk posed by these ticks to humans and animals.

Conclusions

This study evaluated the diversity of different questing tick species and their phenology in Flint Hills ecoregion and the authors describe the presence of three major tick species having similar density distribution with overlap in emergence. Specifically, the increase in adult density of *D. variabilis* in late July will be a helpful guide for understanding regional and within-herd transmission dynamics of bovine anaplasmosis. The information described in this study also provides a foundation for further study and monitoring of tick population dynamics in this important ecoregion of the Midwest. Further research is warranted in the year-to-year variation in the emergence of the tick as well as the ability of the ticks within a region to harbor the disease without continued reinfection from reservoir cattle.

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Tables

Table 2.1 Primer and probes used in Real-time PCR assay for species confirmation on ticks collected in the Flint Hills ecoregion (USA) of Kansas between March 2015 and March 2017.

Species	Target gene	Product size	Primes /probes	Sequence (5'-3')
Amblyomma americanum	16S rRNA	150bp	*FP *RP *Pr	TTTAATTGGGGCGATTTAACTA CATCGAGGTCGCAAACTATT FAM-GAACCGTTATTAACGGACACTTGGA-BHQ-1
Amblyomma maculatum	16S rRNA	178bp	*FP *RP *Pr	AAGGACAAGAAGACCCTAAGAATTT ATTACGCTGTTATCCCTAGAGTATTT CAL Fluor Red 610-TGAAATTTTTTAATTGGGGCGA-BHQ-2
Dermacentor variabilis	16S rRNA	138bp	*FP *RP *Pr	TGGTATTTTGACTATACAAAGGTATT CCTTAATTTTAATAATTGTTTCTTCAC CAL Fluor Gold 540-TGCTAAGAGAATGGAATTACAGGGAATA-BHQ-1

*FP: Forward primer; RP: Reverse primer; Pr: Probe.

Table 2.2 Number of ticks collected from Flint Hills ecoregion (USA) over a two-year study period between March 2015 – March 2017 and categories.

Species	Year	Male	Female	Nymph	Larvae	Total
Amblyomma americanum	Year 1	518	782	1265	1309	3874
	Year 2	506	887	1447	1369	4209
Amblyomma maculatum	Year 1	222	269	-	-	491
	Year 2	203	271	-	-	474
Dermacentor variabilis	Year 1	321	450	-	-	771
	Year 2	286	424	-	-	710
Ixodes scapularis	Year 1	2	6	-	-	8
	Year 2	5	15	-	-	20

Table 2.3 Associations of climate and land cover properties with density of *Amblyomma americanum* ticks (larva, nymph and adult together) fitted in a generalized linear mixed model (glmm).

Covariate	Estimate	Std. Error	Pr (>|z|)	95% CI
Accumulated temperature	1.232	0.012	0.000	1.209, 1.256
Photoperiod	1.079	0.031	0.000	1.019, 1.140
NDVI	0.121	0.010	0.017	0.101, 0.1406
Location	1.553	0.496	0.015	0.580, 2.525
Month	0.811	0.213	0.007	0.039, 1.228

Table 2.4 Associations of climate and land cover properties with density of adult *Dermacentor variabilis* ticks fitted in a generalized linear mixed model (glmm).

Covariate	Estimate	Std. Error	Pr (>|z|)	95% CI
Accumulated temperature	1.421	0.119	0.000	1.877, 1.654
Photoperiod	1.549	0.088	0.001	1.376, 1.721
Saturation deficit	-1.388	0.192	0.038	-1.764, -1.011
Location	1.321	0.622	0.048	0.101, 2.540
Month	0.422	0.183	0.001	0.063, 0.780

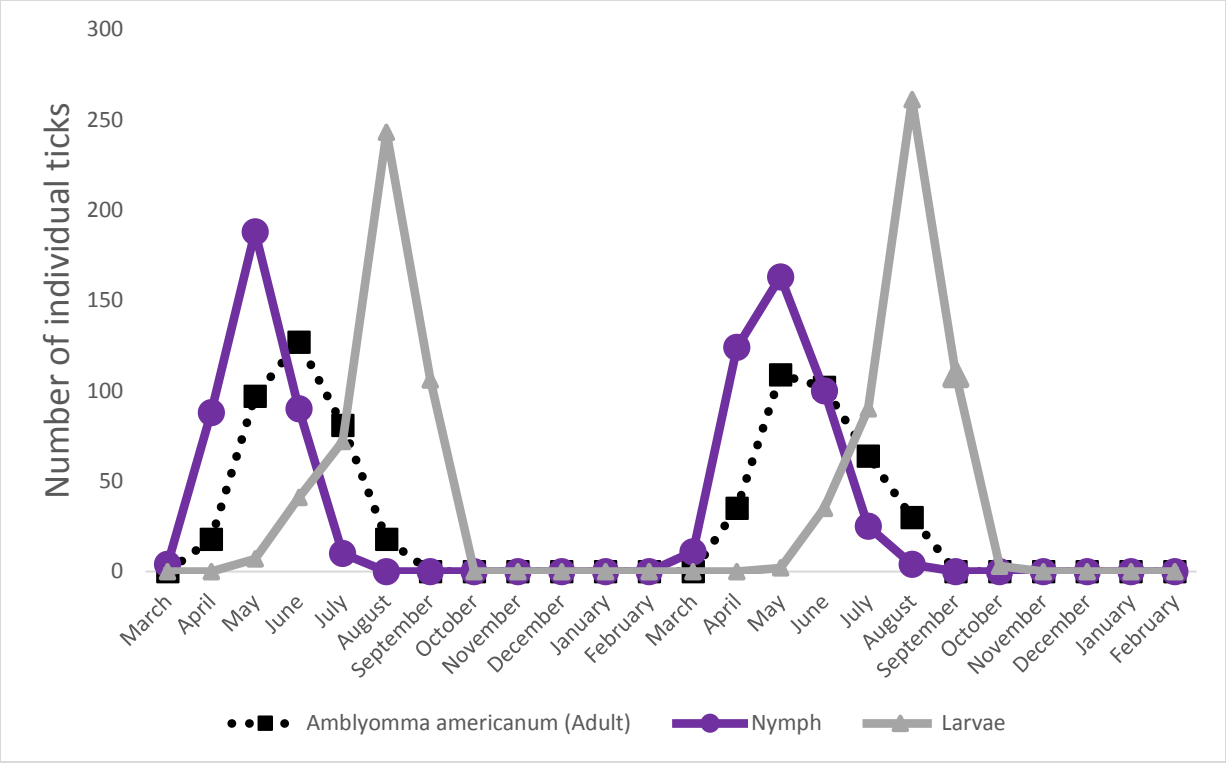
Table 2.5 Associations of climate and land cover properties with density of adult *Amblyomma maculatum* ticks fitted in a generalized linear mixed model (glmm).

Covariate	Estimate	Std. Error	Pr (>|z|)	95% CI
Accumulated temperature	2.076	0.083	0.000	1.913, 2.238
Photoperiod	0.373	0.108	0.000	0.165, 0.588
Mean relative humidity	-0.274	0.187	0.041	-0.640, 0.092
Location	1.118	0.288	0.038	0.553, 1.682
Month	1.027	0.261	0.001	0.514, 1.538

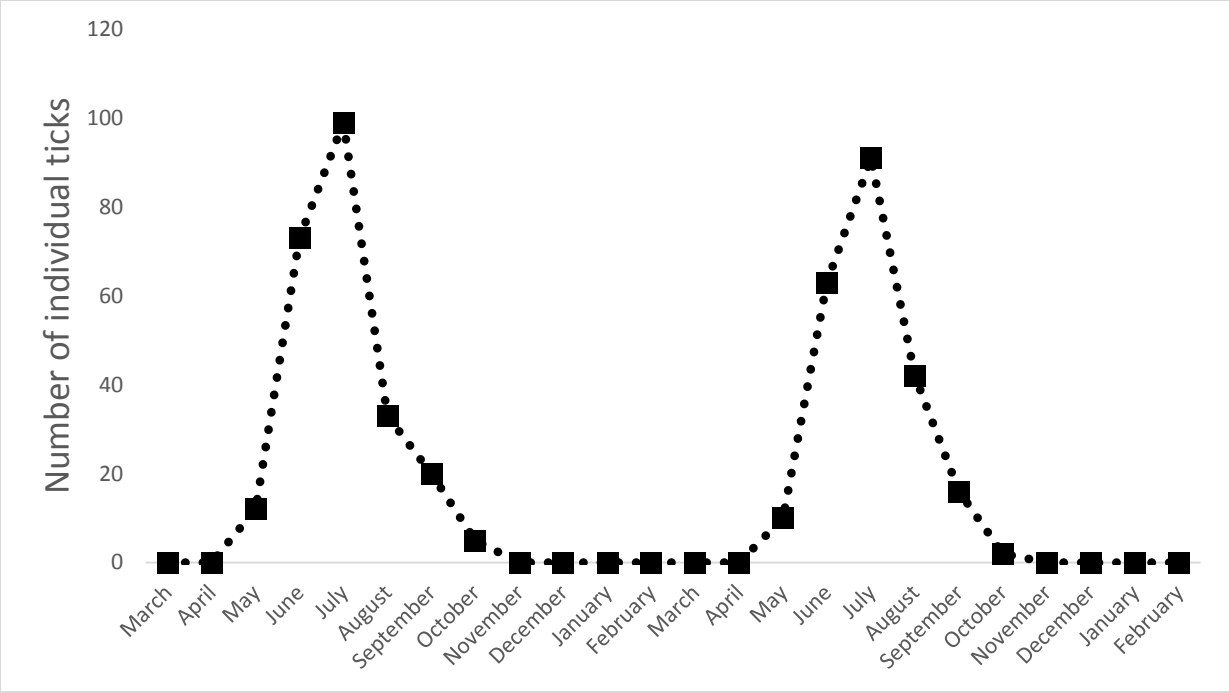
Figures

Figure 2.1 Number of ticks collected every month from sampling locations in Flint Hills ecoregion (USA). A) *Amblyomma americanum* adult, nymph and larvae; B) *Dermacentor variabilis* adults; C) *Amblyomma maculatum* adults.

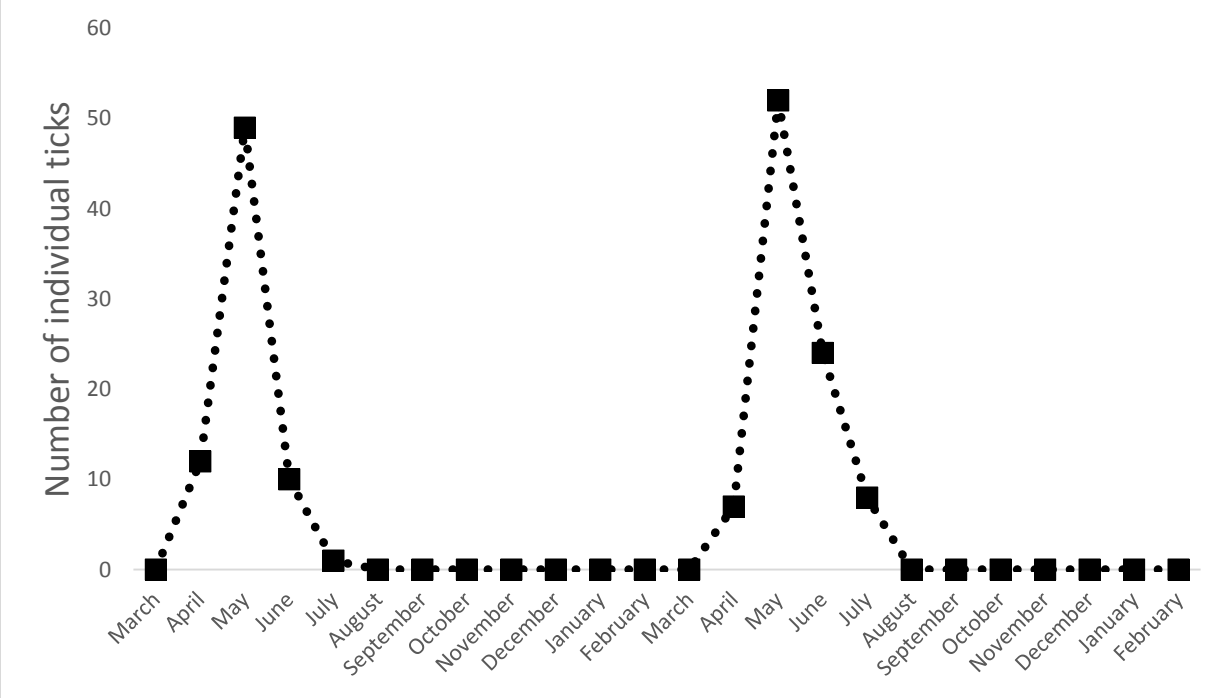
A) *Amblyomma americanum*



***B) Dermacentor variabilis (*adults)**



C) ***Amblyomma maculatum*** **(adults)**



Chapter 3 - Bovine Anaplasmosis Herd Prevalence and Management Practices as Risk-Factors Associated with Herd Disease Status.

Abstract

Bovine anaplasmosis is a hemolytic disease of cattle caused by *Anaplasma marginale* which can cause anemia, adult mortality, abortion, and performance reduction. The objectives of this study were to estimate herd-level infection prevalence of bovine anaplasmosis in Kansas cow-calf herds and assess management practices associated with herd infection status. Licensed Kansas veterinarians were randomly selected and provided clientele to generate randomly selected participant herds. Blood samples were collected from 10 mature cows during processing of 925 herds between October 1, 2016 and March 1, 2017. A management survey was completed by 780 herd-owners. Sample status was determined by competitive enzyme-linked immunosorbent assay (cELISA); operations indicating vaccination for anaplasmosis were tested with *A. marginale*-specific polymerase chain reaction (PCR). Survey data underwent logistic regression analysis for calculation of odds ratios (OR) and confidence intervals (CI). The herd-level prevalence was 52.5% of cow-calf herds. Prevalence ranged from 19.1% of herds in Western Kansas to 87.3% of herds in Eastern Kansas. Vaccinated herds were more likely (OR = 2.38; CI = 1.16–4.85; $p = 0.02$) to be positive compared to non-vaccinated herds, and herds that utilized insecticide ear-tags were more likely to be positive (OR = 1.9; CI = 1.42–2.55; $p < 0.01$) compared to herds which do not. Operations that prescribe-burned 21-50% and >50% of their pastures were more likely to be test positive, OR = 5.74 (CI = 3 .14–10.51; $p < 0.01$) and OR =

4.78 (CI = 2.33–10.17; $p < 0.01$), respectively, than operations that prescribe-burned <20% of their pastures. In summary, anaplasmosis is present across Kansas beef herds at varied prevalence levels and selected management practices were found to be associated with herd infection status.

Abbreviations

cELISA - competitive enzyme-linked immunosorbent assay

PCR - polymerase chain reaction

OR - odds ratio

CI - confidence interval

Introduction

Bovine anaplasmosis is a hemolytic disease of cattle caused by the bacterium *Anaplasma marginale* which can cause adult mortality, abortion, weight loss, and a reduction in performance (Howden et al., 2010; Kocan et al., 2010; Kocan et al., 2003). The disease is common throughout tropical and sub-tropical regions of the world having widespread economical significant distribution throughout much of the United States (Kocan et al., 2010; Kocan et al., 2003). Transmission to susceptible animals occurs through a variety of mechanical vectors, such as flies and veterinary instruments, and biological vectors, such as some tick species (de la Fuente et al., 2003; Eriks et al., 1989; Ewing et al., 1997; Kuttler and Simpson, 1978; Lankester et al., 2006; Scoles et al., 2005; Stewart, 1979). Cattle that survive infection become persistently infected carriers which serve as the reservoir for naïve bovines (Aubry and Geale, 2011). Due to the

subclinical nature of persistently infected animals, some producers are unaware of the infection status of their herd. This unknown infection status can impair the ability of cattle producers and veterinarians to design anaplasmosis control programs. Many studies have investigated the prevalence of *A. marginale* in several US states, but no randomized study to assess statewide prevalence has been completed (Alderink and Dietrich, 1983; Hairgrove et al., 2015; Hairgrove et al., 2014; Morely and Hugh-Jones, 1989; Utterback et al., 1972; Zaugg and Kuttler, 1985). The objective of this study was to estimate herd-level infection prevalence in cow-calf herds and assess management practices associated with herd infection status in Kansas.

Materials and Methods

Study design

Kansas cow-calf herd number and herd size inventory estimates for each agricultural district, provided by the National Animal Statistics Service (NASS, 2012) were used to calculate the number of herds required to estimate agricultural district herd prevalence using an Ausvet EpiTools on-line calculation tool (Ausvet, 2016). Herd prevalence estimates entered into the program included 10%, 20%, and 30% for the Western, Central, and Eastern districts, respectively.

Because a list of all cow-calf operations in Kansas was not available, a sampling frame of all Kansas licensed veterinarians in each district was used to enlist beef cattle herds into the study. Veterinarians practicing within each district were randomly selected to participate in the study using a random number generator from the Stata program (Stata version 14, 2016). The number of practitioners to include into the study was estimated assuming each veterinarian would have approximately 10 participating herds. Kansas State University veterinary students then contacted each randomly selected veterinarian. Those practitioners who stated they were not

cow-calf veterinarians (e.g. small animal only, retired, industry, etc.) or not willing to participate, were eliminated from the study. Participating practitioners were asked to compose a list of 20 producers they believed would be interested in participating in the study, and whose herd contained at least 10 adult beef cows. The veterinarians were asked to assign each producer a unique number between 1 and 20. From the list of producer numbers for each practitioner, researchers selected herds to participate using a commercial random number program from Microsoft Excel (Excel, 2016). The veterinarians were then instructed to select 10 mature animals using a sampling strategy provided by the researchers. In this strategy, 10 head of the first 20 mature females processed were chosen for sampling in alternating fashion. At initiation of the study, the researchers performed a single coin-flip to select either the first or second mature cow through the working facility with which to start sampling. After the selection of either the first or second mature animal to start the selection, every other animal through the working facility was sampled until 10 total samples were collected.

Samples

Herd sampling was targeted during the period October 1, 2016 to March 1, 2017. The targeted period was selected to allow samples to be collected during other cow processing procedures (e.g. transrectal pregnancy diagnosis) and to reduce the possibility of recent vector transmission. The sample collection and survey portions of this study were conducted in accordance with the Kansas State University Institutional Animal Care and Use Committee protocol #3815. Practitioners were provided necessary supplies including syringes, needles, packing supplies, and shipping coolers by the researchers. Samples included blood collected by tail vein into a 10 ml serum tube (BD Vacutainer Glass Serum Tube, 10 ml) and a 3 ml whole blood tube containing ethylenediaminetetraacetic acid (EDTA) (BD Vacutainer Glass Whole

Blood Tube, 3 ml) from each selected animal. Veterinarians were asked to refrigerate the samples immediately after collection and samples were submitted weekly to the Kansas State Veterinary Diagnostic Laboratory. Both serum and whole blood were stored at -20°C.

Testing procedures

At the completion of the collection period, serum samples were delivered to a commercial laboratory for *A. marginale* competitive Enzyme Linked Immunosorbent Assay (cELISA) testing using the Anaplasma Antibody Test Kit version 2, (VMRD, Pullman, WA) Polymerase chain reaction (PCR) was completed at the Kansas State College of Veterinary Medicine on whole blood samples from herds reporting the use of *A. marginale* vaccine.

Survey

A management survey was administered to each producer by the veterinarian at the time of sample collection. The survey contained 41 closed and 3 open-ended questions regarding herd demographics, biosecurity, health management, parasite management, pasture management, and anaplasmosis knowledge.

Statistical analysis

Data were entered into a commercial spreadsheet program (Excel, 2016) and evaluated for accuracy. All data were then imported for analysis into a commercial statistical software program (Stata, 2016). The outcome variable of interest for each operation was herd anaplasmosis infection status. Each independent variable was initially assessed in univariable models. Independent variables were retained for further analysis when the *P*-value for an unconditional association was ≤ 0.30. Variables that remained following the univariable analyses were entered into a multivariable model, and manual backward selection was used to select independent variables that were significantly ($P< 0.05$) associated with the outcome variable.

Each variable that was not retained during the initial backward elimination process was later reoffered to the model to reassess significance and check for confounding. Any variable reoffered to the final model that was significant or resulted in a coefficient change greater than 20% for any other variable was retained in the model. This process continued until no variables reoffered to the model were eligible for retention. Unique veterinarian number was retained in each model because it was considered to be a possible confounder. Odds ratios were chosen for the final model due to their fit with the study objectives to describe associations relative to reference measures at a point-in-time. The odds ratio (OR) represents the odds that an outcome will occur relative to a baseline or reference measure. In the present study, a positive OR represents an outcome is more likely to be found true compared with the reference outcome associated with a particular management practice, and a negative OR would indicate that an outcome is less likely to found true when compared with the reference outcome. A 95% confidence interval was used in the present study.

Results

The sampling frame included 1,483 Kansas licensed veterinarians. A total of 164 licensed veterinarians participated in the study. (Figure 1) The number of veterinarian participants by NASS defined district averaged 18.2 (range 9 to 31). In total, 925 herds participated in the prevalence portion of the study, and 780 (84.3%) participated in both the prevalence and survey portions of the study. The number of cow-calf operations that participated in the study averaged 102.7 per district (range 61 to 153). (Table 1) The average number of sampled herds per veterinarian in each district ranged from 3.5 herds to 10.1 herds.

In total, 925 Kansas cow-calf operations (9,250 mature cows) were sampled. Overall 52.5% (486/925) of cattle herds were found to be *A. marginale* cELISA test positive. The largest

test prevalence risks were found in the three eastern Kansas agricultural districts including 78.2%, 76.9%, and 87.4% for the Northeast, East Central, and Southeast districts, respectively. (Figure 2) The smallest prevalence risks were found in the western districts and included 19.8% in the Northwest, 19.1% in the West Central, and 34.4% in the Southwest districts. Central Kansas district prevalence risks were 44.2%, 57.3%, 46.4% in the North Central, Central, and South Central districts, respectively. (Figure 2 and Table 2)

Of the sampled herds, 4.8% (45/925) reported vaccinating for anaplasmosis. Vaccination use was reported in each district except the North Central district, and 42% (19/45) of herds indicating vaccine use were located in the Southeast district. Of the vaccinated herds 75.6% (34/45) were cELISA positive and 73.3% (33/45) were PCR positive. All vaccinated herds that were PCR positive were also cELISA positive, and one cELISA positive herd was PCR negative. Vaccinated herds found to be PCR positive were designated as infection positive herds for the multivariable model. The overall infection prevalence in the study was 51.7% (474/925). (Table 2)

Survey results

Of the 925 sampled herds, 780 producers completed the accompanying management survey and were included in the risk analysis. The average reported herd size was 189 adult animals (range 10 to 2,000). The respondent herd size frequencies are reported in Figure 3 and the number surveys completed in each district are reported in Table 1. Breed composition included, 76% (593/780) British influence, 7.2% (56/780) Continental, and 14.6% (111/780) mixed breeds. The operation types represented were 85.6% (668/780) commercial, 3.2% (25/780) purebred, and 11.1% (87/780) were both types. Targeted calving period of the respondent herds included 74.4% (583/780) spring calving (January – July), 2.7% (21/780) fall calving (August – December), and 22.6% (176/780) targeted both time periods. Of the respondents, 97.8% (763/780) reported having a general vaccine program in place, and 96.2% (756/780) indicated that their veterinarian was an advisor in the development of their vaccine program. Reported yearly vaccine use consisted of 12.7% (99/780) of herds which used two or fewer vaccines and 77.3% (681/780) herds which used three or more vaccines. Of the respondents, 2.6% (20/780), 17.1% (133/780), 33.1% (258/780), 30.6% (239/780), and 16.7% (130/780) reported using 0, 1, 2, 3, or four or more parasiticides during the year. Thirty respondent producers (3.9%) reported changing needles between every animal when administering injections to cattle during processing or treatment. Of the operations that implanted cattle, 15.9% (124/780) indicated the implant gun was disinfected between each animal. Of the operations utilizing permanent tattoos, 6.0% (47/780) reported disinfecting the tattoo gun before use on a subsequent animal. Of the operations that castrate bull calves, 35.4% (276/780) banded at birth, 15.4% (120/780) banded at weaning, and 57.1% (445/780) castrated by knife, of which 49% reported disinfecting the surgical tool between animals. Only 55.3%

(431/780) of the producers answered questions concerning chlortetrycline use. Chlortetracycline was reported to be used by 25.3% (109/431) of respondents and 19.3% (83/431) reported year-round use of chlortetracycline while 6.0% (26/431) only used chlortetracycline in the spring and summer months.

Independent variables were assessed initially in a univariable analysis. (Table 3) Variables from questions that were answered by <5% of the study participants were excluded from the analysis. These variables included diagnostic testing prior to new cattle entering the herd, type of cattle imported, and targeted consumption of chlortetracycline.

Several variables of interest were not associated *(P* > 0.30) with the outcome of interest. Herd characteristic variables such as breed (Continental, British, or British X Continental), herd type (cow-calf only, cow-calf with feeders, cow-calf with stockers), and operation type (commercial, registered, or both) were not associated with herd infection status for anaplasmosis. Origination of cattle from United States geographical regions Northeast, Southeast, North Central, South Central, Midwest, and West United States was not associated with anaplasmosis status of the Kansas cow herds. Health management variables such as disinfection of castration knife or implant gun, importation of cattle, or testing cattle for anaplasmosis were not associated with herd infection status. Parasite control measures such as the inclusion of a mineral insect growth regulator, the use of an injectable dewormer, or the use of electronic direct fly control were not associated with cow herd anaplasmosis status.

The final multi-variable model included three variables associated with herd infection designation *(P* < 0.05). (Table 4) Compared with herds that do not use insecticide ear tags, herds which utilized insecticide ear tags were more likely to be anaplasmosis positive *(P* < 0.01). Herds which utilized an anaplasmosis vaccine were more likely to be test positive *(P* = 0.02)

compared with herds which do not vaccinate for the disease. Compared with herds which prescribe burn <20% of pastures, operations which prescribe-burn 20%-50% and operations which prescribe-burn greater than 50% of pastures had increased risk ($P < 0.01$) of a positive herd status.

Discussion

According to Aubry and Geale, the prevalence of bovine anaplasmosis in the US is largely unknown, and accuracy in published test prevalence or incidence reports is challenging because of the difficulties in executing population-based or random sampling (Aubry and Geale, 2011). The current study reported the percentage of tested herds positive for anaplasmosis of 51.7%. The present study was not a completely random sampling of Kansas herds, but was limited to the existing infrastructure of existing veterinary-client relationships in the state. Veterinarians were randomly selected to participate, but because they were asked to provide a list of producers who potentially would be willing to participate this may have injected bias into the study. It is plausible that only those producers who have a strong working relationship with the practitioner or utilize the practitioner for routine services (i.e. pregnancy examination) had the potential for participation. It is also possible veterinarians listed those herds that were suspected, but not confirmed, as anaplasmosis positive (i.e. for Veterinary Feed Directive information) or listed herds familiar with and concerned about anaplasmosis. Therefore these herds may or may not represent the average Kansas cow-calf operation. The prevalence estimate generated in the current study was likely affected by the study design's limitations concerning the ability to detect disease within each herd. The calculated herd sensitivity for sampling 10 mature cows in herds with lower within- herd prevalence may have resulted in some herds misclassified as negative. For instance, calculated herd sensitivity for herds with 10% within-

herd prevalence were 69%, 67%, 66% for herd sizes of 50, 100, and 500, respectively (Ausvet, 2018). The prevalence estimates from most other studies sampled cattle within a state were on an individual and not herd basis. Utterback and others in 1969-1970 reported 43% (3,519/8,156) of the cattle they tested were positive in California using the Complement Fixation test (Utterback et al., 1972). These samples were collected over separate periods during 1969 and 1970 and using a combination of serum collected at slaughter and practitioner submissions (Utterback et al., 1972). Zaugg and Kuttler in 1985 reported 12.6% (1,283/10,167) individuals and 29.17% (119/408) herds they sampled were infected in Idaho (Zaugg and Kuttler, 1985). These samples were collected over a period of two years and obtained the samples using a combination of serum from regulatory testing, practitioner submissions, and regularly sampled herds (Zaugg and Kuttler, 1985). Animals classified as positive for anaplasmosis infection were positive on both Rapid Card Agglutination test and Complement Fixation test (Zaugg and Kuttler, 1985). Morely in 1985 reported 7.8% (860/11,085) of individuals and 58.9% (123/209) herds were test positive in Louisiana (Morely and Hugh-Jones, 1989). The samples in the study were from 14 parishes representing the Red River Plains areas and the Southeast area of Louisiana amounting to 29% of the state's beef cow population and 76% of the state's dairy cow population. Sample collection in the Morley study consisted of two serum banks collected under the state's Brucellosis Eradication Program during the years 1982 and 1984; testing was conducted using the Indirect Fluorescent Antibody test (Morely and Hugh-Jones, 1989). In 2014, Hairgrove and others reported a pooled apparent seroprevalence of 15.02% using a commercial cELISA test in 1,835 serum samples collected from 23 livestock auction markets across the state of Texas in July of 2011 (Hairgrove et al., 2014). More recently, in 2015, Hairgrove and others reported 40.1% (174/434) of individual cattle they sampled in 11 Texas herds were test positive representing

regions tested in their auction market study (Hairgrove et al., 2015). These studies' estimates may be limited by the lack of a random sampling study design. Many of the samples for these studies were obtained through the collection of serum for the surveillance of other diseases (Morely and Hugh-Jones, 1989; Utterback et al., 1972; Zaugg and Kuttler, 1985). Differences between those studies' estimates and the present study may be explained by the difference in targeted sampling populations, including management differences between dairy and beef operations, and practitioner involvement in sample collection. Additionally, some of the previous studies may have used diagnostic tests with poor diagnostic sensitivity and may have resulted in under-reporting true herd prevalence (Bradway et al., 2001; OIE, 2015).

The use of insecticide ear tags was the only parasite control method with statistical significance (OR = 1.9, $P < 0.01$) remaining in the final model indicating an association with herd infection designation. Interestingly no association was noted with herd infection with regard to the number of varied parasite control methods used. This finding could indicate that insecticide ear tag application shares a geographic distribution with areas of higher concentrations of anaplasmosis infected herds. It could also be that insecticide ear tags are commonly applied in areas of elevated ectoparasite burdens, and those ectoparasites contribute to the transmission of anaplasmosis and maintenance of positive herds in the region. Thus increased insecticide ear tag application may be a response to increased risk, and not necessarily infection status.

Whole-pasture prescribed burning and patch burning have been suggested to play a role in decreasing the number of ticks exposed to cattle (Polito et al., 2013). Although this study did not find an association between herd status and the presence of ticks in Kansas, Utterback and Zaugg both indicated the presence of this association in their respective studies in California and

Idaho (Utterback et al., 1972; Zaugg and Kuttler, 1985). The investigators in the present study were interested in the frequency of burning as reflected by the percent of grazing area burned in the past three years. Compared with operations which had previously prescribe-burned 0-20% of their pastures, operations which prescribe-burned 20-50% and operations or prescribe-burned >50% of pastures were 5.74 and 4.78 times more likely to have been test positive, respectively. These findings may be due to a distribution of prescribed-burning that is in more common in areas with heavy tick burdens; conversely the findings may be suggestive that pasture burning is not an effective method of tick control. A third plausible explanation to the increased likelihood of infection in areas where burning large areas is practiced includes the possibility these environmental conditions that influence the distribution of range burning also allow for an increased stocking density of livestock. Increased stocking density may also enhance the opportunities for transmission from infected to naïve animals and thereby increase disease prevalence as was found in the study by Utterback (Utterback et al., 1972).

The current study had several opportunities for the injection of bias. Selection bias at the level of the researchers and the veterinarians was possible. Veterinarians were randomly selected to participate, but it is possible that only those veterinarians interested in the distribution of bovine anaplasmosis in their practice area participated. Likewise, producers who agreed to participate may have been motivated to discover the disease status of their herd. Recall bias was also possible in the completion of the survey because some questions asked about management practices occurred in the past. Additionally, cooperator bias may have influenced the participant herds as their veterinary relationship may make them more likely to utilize a veterinarian, perhaps more interested in research, and more likely to utilize progressive management techniques.

Conclusion

The results of the current study reflect a wide distribution of anaplasmosis across most Kansas agricultural districts. This study indicated that some management practices are associated with herd infection status, but many commonly promoted anaplasmosis management practices were not strongly associated with herd anaplasmosis infection. Further studies are needed to examine the economic impact and the molecular and pathogenic variants in the state and across the nation.

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Declaration of Conflicting Interests

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Tables

Table 3.1 – Number of participating veterinarians and cow-calf study herds in Kansas

Agricultural District	Number of Veterinarians	Number of Herds	Surveys Completed
Northwest	12	121	108
North central	17	104	75
Northeast	29	101	86
West Central	9	73	69
Central	13	96	83
East central	31	121	91
Southwest	13	61	47
South central	18	153	141
Southeast	22	95	80
Study Total	164	925	780

Table 3.1 Anaplasmosis herd prevalence results by agricultural district with PCR results included.

Agricultural District	Seroprevalence	Vaccinated Herds	PCR-Positive Herds	PCR-Negative Herds Excluded
Northwest	19.8%	2	0	18.1%
North central	44.2%	0	0	44.2%
Northeast	78.2%	3	2	77.2%
West Central	19.2%	2	0	16.4%
Central	57.3%	3	3	57.2%
East central	76.9%	7	6	76%
Southwest	34.4%	5	3	31.1%
South central	46.4%	4	2	45%
Southeast	87.3%	19	17	85.2%
Overall	52.5%	45	33	51.2%

Table 3.2 Results of univariable logistic regression model indicating management practices that were associated (P ≤ 0.30) with the bovine anaplasmosis herd infection status in 780 Kansas cow-calf operations from data obtained in survey.

Variable	P-value	OR	95% CI of the OR
Herd size	0.03	0.87	0.77 - 0.99
See Ticks on your cattle	<0.01	2.45	1.43 – 4.21
Disinfect castration knife	0.57	1.15	0.69 – 1.92
Disinfect dehorner	0.27	1.44	0.75 – 2.79
Disinfect ear notcher	0.22	1.45	0.79 - 2.65
Implant gun disinfected	0.40	0.78	0.43 - 1.41
Number of parasiticides used	0.05	1.14	1.00 - 1.29
Insecticide eartags used	<0.01	2.53	1.87 - 3.41
Injectable dewormer used	0.92	0.98	0.63 - 1.53
Pour-on dewormer used	0.04	0.34	0.12 - 0.95
Mineral IGR	0.83	1.04	0.73 - 1.49
Backrub bags used	0.06	0.63	.40 - 1.01
Fog	0.05	1.61	1.00 - 2.60
Electronic zappers	0.31	0.47	0.11 - 2.05
Change needles	0.18	1.68	0.79 - 3.59
Vaccine advice	0.15	1.42	0.88 - 2.29
Test for anaplasmosis	0.96	1.03	0.31 - 3.38
Pasture use	0.23	1.20	0.89 - 1.62
Vaccinated	<0.01	3.13	1.57 - 6.26
Import cattle	0.59	1.06	0.86 - 1.29
Use anaplas vaccine	<0.01	3.72	1.58 - 8.77
Hay Purchase	0.13	0.85	0.69 - 1.05
Targeted calving	<0.01	2.53	1.70 - 3.76
Operation type	0.58	1.09	0.79 - 1.50
Herd type	0.99	1.00	0.76 - 1.33
Pasture burn	<0.01	3.08	2.05 - 4.64
Import region	0.19	0.59	0.26 - 1.32
Breed	0.69	1.05	0.84 - 1.31
District	0.01	1.17	1.04 - 1.31
Veterinarian	<0.01	1.02	1.01 - 1.02
CTC use	<0.01	1.83	1.34 - 2.48
Supplement hay	0.10	1.38	0.93 - 2.04
Number of vaccines	0.19	0.92	0.80 - 1.05

Table 3.3 Results of the multivariable logistic regression model indicating management practices that were associated ($P < 0.05$) bovine anaplasmosis herd infection status in 780 Kansas cow-calf operations using a survey.

Variable	P-value	Level	*B*	SE(*B*)	OR	95% CI of the OR
Insecticide eartag	<0.01	No				
		Yes	0.64	0.15	1.90	1.42 - 2.55
Use Anaplasma vaccine	0.02	No				
		Yes	0.87	0.36	2.38	1.16 - 4.85
Pasture burn	<0.01	0-20%				
		20-50%	1.75	0.30	5.74	3.14 - 10.51
		>50%	1.58	0.37	4.87	2.33 - 10.17
Veterinarian	0.24		0.00	0.00	1.00	1.00 - 1.01

Figures

Figure 3.1 Graphical demonstration of the randomization and selection of veterinarian participants from a sampling frame of licensed Kansas veterinarians

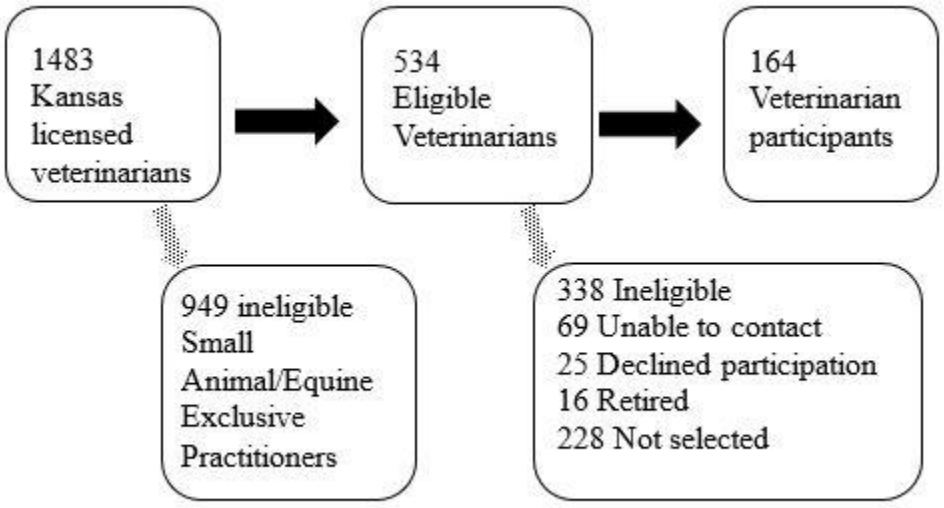


Figure 3.2 Apparent prevalence estimates for the nine agricultural districts in Kansas

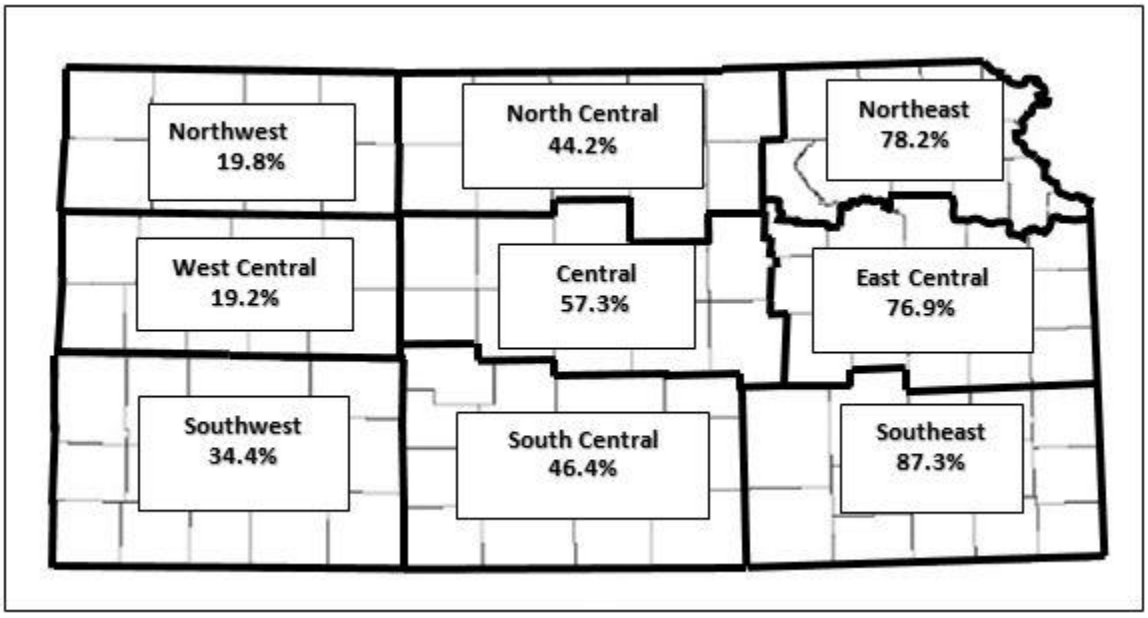
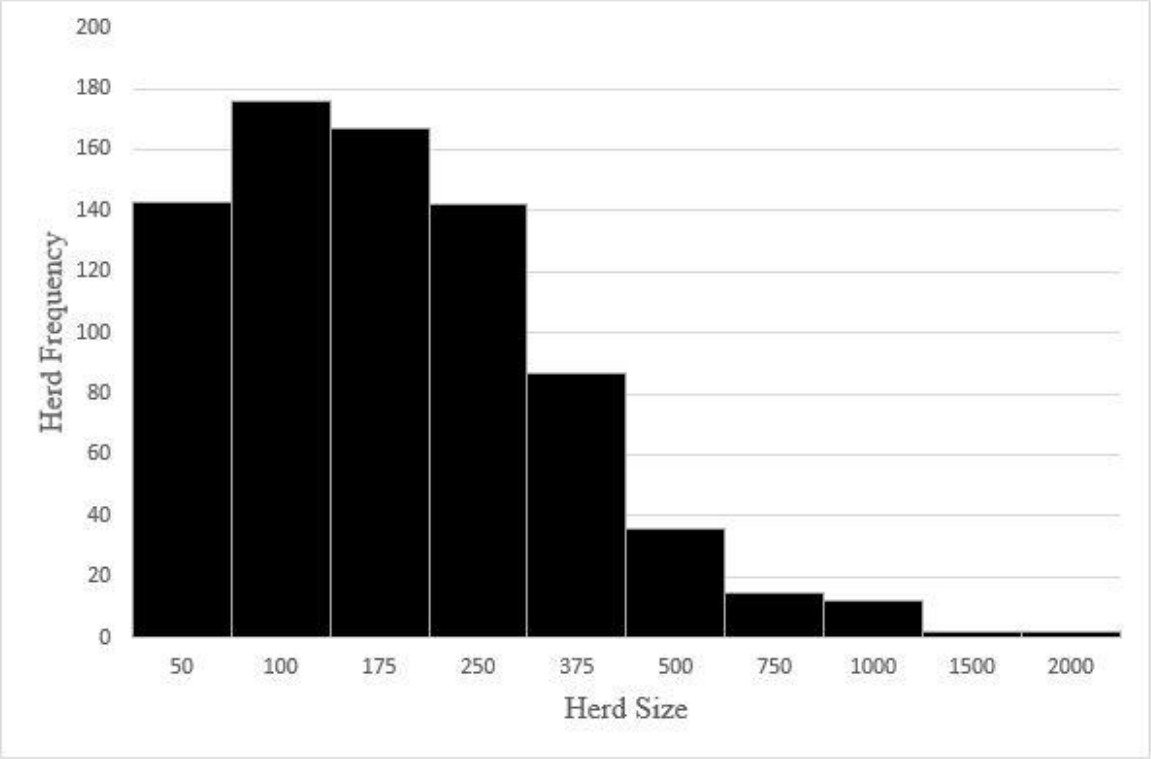


Figure 3.3 Herd size frequency of Kansas Bovine Anaplasmosis survey respondents



Chapter 4 - Administration of in-feed chlortetracycline alone, or in combination with injectable oxytetracycline to mature beef bulls, and observed anaplasmosis infection status

Abstract

Anaplasma marginale causes hemolytic disease in cattle and few approved treatment options exist for infected animals. Yearling bulls (n=827 hd) were used to examine if CTC alone, or in combination with injectable OTC, could clear apparent anaplasmosis infection. Blood samples were obtained to determine anaplasmosis infection and seroconversion status in bulls on day (D) 0. All bulls received CTC in their diet for 80 days. Thirty-eight bulls were anaplasmosis positive on D 0 and half of them received OTC on D 40. Blood samples were taken from anaplasmosis positive bulls on D 40, 80 and 128 for *A. marginale* diagnostics. Neither CTC alone nor in combination with OTC had detectable effects on clearing bulls with high reported percent inhibition for anaplamosis using cELISA. Oxytetracycline injection resulted in decreased ADG in bulls. All anaplasmosis positive bulls with low reported percent inhibition on D 0 were anaplasmosis free and cELISA negative by the end of the feeding period when fed CTC. No bulls with high reported percent inhibition were cleared of anaplamosis. Bulls not as seronegative by D 40 were never seronegative during the study. Lower reported percent inhibition values were predictive of which naturally-infected bulls would be cleared from anaplasmosis with CTC feed therapy.

Key words: anaplasmosis, beef cattle, therapy

Abbreviations

CTC – chlortetracycline

OTC – oxytetracycline

cELISA - competitive enzyme-linked immunosorbent assay

PCR - polymerase chain reaction

CI - confidence interval

Introduction

Bovine anaplasmosis is a hemolytic disease of cattle caused by the bacterium *Anaplasma marginale* which can cause adult mortality, abortion, weight loss, and a reduction in performance (Howden et al., 2010; Kocan et al., 2010; Kocan et al., 2003). The disease is common throughout tropical and sub-tropical regions of the world having widespread economical significant distribution throughout much of the United States (Kocan et al., 2010; Kocan et al., 2003), including a statewide presence in the state of Kansas, (Spare et al, 2020). Transmission to susceptible animals occurs through a variety of mechanical fomites, such as flies and veterinary instruments, and biological vectors, such as some tick species (de la Fuente et al., 2003; Eriks et al., 1989; Ewing et al., 1997; Kuttler and Simpson, 1978; Lankester et al., 2006; Scoles et al., 2005; Stewart, 1979). Cattle that survive infection become persistently infected carriers and then serve as the reservoir for naïve cattle (Aubry and Geale, 2011). Due to the subclinical nature of persistently infected animals, some producers are unaware of the infection status of their herd. This unknown infection status can impair the ability of cattle producers and veterinarians to design anaplasmosis control programs. Recently, efforts have been made to compare drug delivery strategies for groups and the pharmacodynamics of group feeding which make modern large-scale feed delivery desirable (Schrag et al, 2020). Existing literature includes reports of

attempts to clear the disease from the individual animal with therapies of varied drug formulations, concentrations, routes, and durations. Results of these studies have been mixed, with successful chemosterilization reported historically by feeding chlortetracycline 3.3 to 11 mg/kg for 30 to 60 days (Brock et al., 1959; Franklin et al., 1965, 1966, 1967; Richey et al., 1977; Sweet and Stauber, 1978; Magonigle and Newby, 1983; Reinbold et al, 2010) yet others reported failing to achieve clearance (Kuttler et al, 1980; Coetzee et al, 2005). Many of these studies used diagnostic tests that are no longer used today due to poor sensitivity and/or specificity. Therefore, the purpose of this study was to evaluate the ability of long-term feeding of approved concentrations of chlortetracycline alone, or in combination with a supplemental injection of long-acting oxytetracyclne, to clear anaplasmosis infection and cELISA serology status in naturally infected mature beef bulls in a commercial setting.

Materials and Methods

The current study took place between August 27, 2019 and April 12, 2020. The majority of this period fell outside the vector season in Kansas. Yearling bulls (n=827, BW= 501.6 +/- 61.4 kg, age = 360 days +/- 20.9 days) located in confined bull development facility in western Kansas were used to evaluate the ability of long-term feeding of approved concentrations of chlortetracycline alone, or in combination with a supplemental injection of long-acting oxytetracyclne, to clear anaplamosis infection and cELISA serology status in naturally infected mature beef bulls. The genetic background of all bulls was purebred Black Angus and all bulls were raised with the intent for market as breeding animals. The bulls were assembled at the facility following separation from their dams and were fed a growing diet prior to the study. This study was approved by Kansas State University Institutional Animal Care and Use Committee, protocol number 4316.

Initial Sampling

On Day -10, during routine ultrasound scanning procedures of all bulls, a 12-ml blood sample was collected from each animal via coccygeal vein using a 16 gauge x 5/8" needle. Then, 3 ml of blood was immediately transferred to K2EDTA tube and 9 ml to a serum separator blood tube. Samples were stored at 4 degrees Celsius while they were transferred to the laboratory where all samples were centrifuged, harvested and then stored at -20 degrees Celsius until serum samples could be submitted to Kansas State Veterinary Diagnostic Lab and analyzed for the presence of antibodies to *Anaplasma marginale* using a competitive enzyme-linked immunosorbent assay (cELISA[a]).

Serial Monitoring

Bulls determined to be positive (n = 38 hd) were monitored during the second portion of the study, and remained housed in their home pen with their contemporary group. Blood samples for serology were obtained on D 40, D 80, and at the time of marketing (D 128, D 178, D 218, and D 234). On D 40 and D 80, the bulls were removed from their pen, walked to the processing facility, sampled for serum and whole blood, weighed and returned to their home pen. The final samples were collected prior to market during pre-sale breeding soundness exam for the individual bulls at D 128 (9 bulls), D 178 (13 bulls), D 218 (7 bulls) and the latest sample at D 234 of the study (6 bulls). Whole-blood samples were analyzed for the presence of *A. marginale* following this final sample collection to confirm status.

Feeding and housing

The bulls were housed in outdoor dirt floor pens consisting of pipe rail fences, solid concrete bunks with concrete feed apron, and bulls had *ad libitum* access to automatic waterers. All bulls (average pen size was 37.6 bulls/pen) received the same grower diet for the duration of

the study (Table 1). The researchers included a measured dose of 1.1 mg/kilogram of bodyweight of chlortetracycline (CTC[b]) daily included in the total mixed ration (TMR) based on the average initial weight in the pen at enrollment, 501.6 kilograms. The dose was increased every 15 days during the feeding to account for the weight gain of the bulls. These increases accounted for an estimated 3 pounds or 1.4 kg of daily gain and equated to a dose increase of 22.5 mg of medication each feeding period. Feed was delivered twice daily and the medication was included in the first feeding each day.

Injectable therapy

On Day 40, seropositive bulls (n=38) were randomized within respective pen and allocated to one of two treatments: 1) injectable oxytetracycline[c] (OXYTET) or 2) no oxytetracycline injection (CON). Each bull was weighed individually and treatment bulls (n=20) were administered a dose of 19.8 mg/kg subcutaneously in the neck per label direction with no more than 10 ml per injection site.

Statistical analysis

Continuous variables were analyzed for both treatment and status change using the LSMeans package in R statistical software (R Core Team (2019), Vienna, Austria). Individual bull was considered the experimental unit for all variables. Treatment least-squares means with P-values ≤ 0.05 were considered different; P-values between 0.05 and 0.15 were considered a trend.

Results

Demographics of the bulls positive or negative for anaplasmosis utilized in this study are described in Table 2. In all, 38 of the 827 bulls (4.6%) were positive for anaplasmosis signified by elevated cELISA status. The proportion of bulls born due to embryo transfer was 60.8%,

meaning that 39.2% of bulls were conceived by natural service or artificial insemination. Embryo transfer derived bulls comprised 66.3% and 55.3% of the anaplasmosis negative and positive bulls, respectively. Overall, the average weight at initial processing for bulls utilized in the study was 501.6 kilograms, and the average age of bulls at study enrollment was 360 days. The average enrollment body weight was 503 kilograms pounds for anaplasmosis negative bulls and 479 kilograms for anaplasmosis positive bulls on D0. The average age for both anaplasmosis positive and negative bulls was 360 days old at D0. Diagnostic results from bulls screened which were found to be anaplasmosis negative averaged -4.9 percent inhibition (%I) and anaplasmosis positive bulls averaged 56.7 %I on the cELISA test.

Further description of the population based on prevalence of anaplasmosis-positive bulls is listed in Table 3. Overall, 4.6% (38/827) of bulls screened were positive for anaplasmsosis. Of bulls born of embryo transfer, 3.72% (21/565) were anaplasmosis positive and 6.58% (17/262) of non-embryo transferred derived bulls were anaplasmosis positive. Bulls 330 days of age or less at initial screening exhibited an anaplasmosis positive prevalence rate of 6.67% (5/75) whereas 4.38% (33/754) of bulls greater than 330 days of age at screening were positive for anaplasmosis. Bulls which were less than or equal to 386.4 kilograms at study enrollment were found to be 12.0% (6/50) positive for anaplasmosis and bulls that weighed more than 386.4 kilograms at enrollment were 4.12% (32/777) anaplasmosis positive.

Oxytetracycline therapy results

Supplemental oxytetracycline (OTC) treatment had no impact on anaplasmosis serology status of naturally infected bulls as illustrated in Figure 1. One animal within each supplemental OTC treatment reverted from positive to negative and back to positive anaplasmosis status during the study. There were no differences between anaplasmosis positive cattle treated with

OTC for initial age, weight, first period average daily gain, overall average daily gain, or reported percent inhibition (Table 4) relative to anaplasmosis positive bulls not receiving a supplemental OTC treatment. Bulls that received OTC injection had significantly lower average daily gain ($p < 0.01$) for the 40 days following administration than bulls that did not receive a supplemental OTC treatment.

Anaplasmosis status change

Anaplasmosis status change in naturally infected positive bulls was dependent on the serological status as described by reported cELISA %I at the day of enrollment when fed an approved concentration of CTC. Anaplasmosis positive bulls with an average %I of 39.2 (95% Confidence Interval (CI): 35.0, 43.3) at the time of initial screening converted to negative status after 40 days of being fed CTC while bulls with an average %I of 72.4 (95% CI: 68.8, 75.9) were unchanged regardless of CTC or OTC treatments. Interestingly, status change from D -10 to D 40 was a significant factor in determining final serostatus. Bulls that changed from serologically anaplasmosis positive status to anaplasmosis negative status did so between 0 and 40 days on a CTC supplemented ration. No bulls changed from positive to negative cELISA serological status after 40 days on CTC supplemented feed. Neither age nor body weight at enrollment, D40 body weight, D80 body weight, or average daily gain were predictive of bulls changing from anaplasmosis positive status to anaplasmosis negative status. (Table 5)

Discussion

Prevalence on initial processing

The current study indicated an apparent prevalence of 4.6% among the population of 827 yearling purebred angus bulls sampled. Other studies have reported varied ranges of anaplasmosis prevalence upon entry to feedyards. One study sampled 659 weaned calves sourced

from Iowa using a cELISA upon entry to a commercial Iowa feeding facility and discovered a 15.7% anaplasmosis positive prevalence rate (Coetzee, et al 2010). Another study reported only 1.57% of 5,608 head of yearling cattle sourced from Montana sampled upon entry into an Alberta, Canada feedlot had antibodies to anaplasmosis (Van Donkersgoed et al., 2001). Taken with the current study, these prevalence reports indicate a wide range of infection level among cattle in this age group.

Percent inhibition effect

The current study found naturally infected cattle were converted from positive to negative serostatus for anaplasmosis by delivering a medicated total mixed ration in a commercial setting according the legal limit set by the United States Food and Drug Administration for feeding this size and age of cattle (FDA, 2015). Bull status was confirmed to be positive or negative using PCR upon exit from the study. Briefly, the amount of antigen-antibody complexing is quantified by measuring the optical densities (OD) of the sample well using an absorbance reader. (Chung et al., 2013). The OD of each sample was translated to (%I) by the following formula: %I = 100 (1 − sample OD ÷ mean negative control) (Chung et al., 2013) from which %I ≥ 30%is interpreted to be positive according to the test manufacturer. Bulls which had a higher reported %I at initial sampling were less likely to be converted from positive to negative overall. Another study found this association when they identified infected cattle by antibody level using a complement fixation test. In their study, infected carrier animals were treated with a series of injections of long-acting tetracycline; cattle with the lowest initial antibody levels were converted from positive to negative serostatus between 30 to 60 days after treatment (Magonigle and Newby, 1983). In the injectable study, chemosterilization was confirmed by injecting blood from study animals into splenectomized steers (Magonigle and Newby, 1983). The complement

fixation test has been indicated to have a diagnostic sensitivity of 7.5 to 37.5% (Coetzee et al., 2007). Using a cELISA, Reinbold et al., reported a successful chemosterilization of 100% of steers fed chlortetracycline at 4.4, 11, and 22 mg/kg bodyweight regardless of initial antibody level (Reinbold et al, 2010).

Effect of Oxytetracycline

In the current study, a single injection of long-acting oxytetracycline at Day 40 of the 80-day medicated feeding period resulted in no detectable difference in outcome of the cattle. Similarly, one study examined three injectable oxytetracycline protocols for the chemosterilization of carrier animals and found that none of the three were sufficient for clearance of cattle infected with anaplasmosis (Coetzee et al, 2005). Magonigle and Newby reported successful chemosterilization of cattle with series of four doses over twelve days of injectable long-acting tetracycline at 20 mg/kg bodyweight (Magonigle and Newby, 1983). Another published combination protocol indicated chemosterilization of Holstein steers using a single injection of oxytetracycline and a medicated diet for 40 days (Reinbold et al, 2010). The concentration of medicated feed used in this protocol was 4-times greater than the concentration used in the current study, and would not be allowable under the current regulations set forth by the Veterinary Feed Directive (VFD).

Seroconversion

Study animals that responded in the current study converted from positive to negative status by D40 of the medicated feeding period. Animals that seroconverted from positive to negative were confirmed negative with PCR analysis of whole blood. A study in Oklahoma reported positive results in chemosterilization of ten head of 2 year-old steers and heifers with anaplasmosis by feeding 1.1 mg/kg body weight for 120 days, the same dose that was used in the

current study (Richey et al, 1977). These authors reported a significant difference developed in titer level between medicated and non-medicated groups at day 42 of the study and this difference was maintained for the duration of the study. Their study results were confirmed using splenectomized animals for injection of blood from study animals. Kuttler et al, 1980, observed a similar serological phenomenon in infected animals treated with injectable oxytetracycline tested at 40 days post-treatment and reported no seroconversion between day 40 and day 90 (Kuttler et al., 1980). An injectable protocol was reported to successfully chemosterilize purebred Hereford cows aged 10-15 years using a series of intramuscular injections of long-acting oxytetracycline at a dose of 20 mg/kg (Magonigle and Newby, 1983). These authors also reported seroconversion of the study cattle between day 30 and 60 following treatment. Chemosterilization was demonstrated at 150 days post-treatment by injecting blood from study cattle into splenectomized calves. Successful chemosterilization has been reported by feeding chlortetracycline 3.3 to 11 mg/kg for 30 to 60 days (Brock et al., 1959; Franklin et al., 1966, 1967, Sweet and Stauber, 1978). These studies did not evaluate serology at timepoints to compare to the current study. Contrary to the current study, when evaluating three levels of chlortetracycline therapy, Reinbold reported seroconversion from positive to negative at 18, 54, and 18 days following the 80-day medicated period (Reinbold et al, 2010).

Average Daily Gain effect

In the current study, injectable antibiotics were administered on D40, however, no cattle were converted from positive to negative status following the injection. Cattle that received the dose of injectable antibiotic had a significantly decreased gain performance following the injection**.** The significant difference in average daily gain between groups of animals that received an injection of long-acting tetracycline was unexpected. In the current study, the loss of

performance equated to (-$18.47) over the second 40-day period with a daily cost of $1.90/day. No other published literature was found to address this potential interaction. Further examination of a potential performance loss is warranted.

The current study utilized naturally infected bulls in a commercial feeding setting. Of the initially sampled bulls, 38 head were found to be seropositive for anaplasmosis and were used in the study. The number of bulls found to be initially positive limited the data available. Furthermore, the study animals were fed in a group setting with other bulls which were negative for anaplasmosis initially and may not have received the correct dose every day due to competition for feed and not having *ad libitum* access to medicated feed. Infections which were refractory to antibiotic treatment may have been genetically different from responsive strains, however, this study did not address those potential genetic differences. The bulls presented to the feeding facility in the late spring or early summer of 2019 and were not sampled until August of 2019. Therefore, some of the bulls which were found to be infected at initial sampling may have become infected after entering the facility which would have increased the reported prevalence from the natural prevalence when the bulls entered the feedyard.

Conclusion

A cohort of yearling angus bulls were examined and found to have an infection prevalence of 4.6% of bovine anaplasmosis. Approximately 40% of yearling angus bulls naturally infected with bovine anaplasmosis were chemosterilized after receiving an approved dose of chlortetracycline in a commercial feeding facility for 80 days. Bulls that did not respond by Day 40 of the medicated feeding period, did not respond at any point during the study period. A single injection of long-acting, subcutaneous oxytetracycline at D40 did not appear effective.

Bulls that received the injection experienced a significant decrease in average daily gain following the injection.

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End notes

[a]cELISA, VMRD, Pullman, WA.

[b]Aureomycin 50, Zoetis, 10 Sylvan Way, Parsippany, NJ 07054

[c]LA 200, Zoetis, 10 Sylvan Way, Parsippany, NJ 07054

Declaration of Conflicting Interests

Loneragan GH, served as a paid consultant, and has accepted honoraria and travel support. All activities occurred in excess of 5 years prior to the commencement of this study. All activities were unrelated to the products described herein. The authors declare no other potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Tables

Table 4.1 Composition of a grower diet (DM-basis) fed to purebred Angus yearling bulls during the 80-day medicated period and the period following withdrawal of the medication.

Item	Ration
Ingredient, %	
Dried distiller grain	**12.0**
Old World Bluestem	**10.0**
Straw	**10.0**
Wheat silage	**52.0**
Dry-rolled corn	**15.0**
Micronutrients	**1.0**
Water	**0**
Nutrient Composition	
DM, %	**42.0**
CP, %	**11.5**
NEM, Mcal/cw (calculated)	**73.0**
NEG, Mcal/cw (calculated)	**42.0**
TDN, %	**66.9**
peNDF, %	**25.5**

Table 4.2 Descriptive measures of purebred Angus yearling bulls included in initial sampling for serostatus[1] to bovine anaplasmosis in August of 2019

Variable	Bulls positive for anaplasmosis (SD)	Bulls negative for anaplasmosis (SD)	Total bulls (SD)
Initial bodyweight, kg	1053 (169)	1106 (135)	1103.6 (137.4)
Initial age, days	360 (39.3)	360 (19.5)	360 (20.9)
Percent inhibition[2]	56.7 (17.6)	-4.90 (14.3)	-1.84 (19.7)
Embryo conception[3]	0.553	0.663	0.608
(n)	38	789	827

[1] Status determined according to cELISA on samples obtained Day-10.
[2] Percent inhibition = 100 – ([sample OD x 100]/mean OD of negative control sample)
[3] Pregnancies resultant of embryo transfer in which conception is achieved by artificial insemination within a donor cow and embryos are harvested at Day 7 of pregnancy and transferred to a recipient dam for gestation, parturition, and suckling.

Table 4.3 Seroprevalence[1] of Bovine anaplasmosis overall and among sub-populations of purebred Angus yearling bulls by embryo class, age, and weight at initial processing.

Factor	Seropositive	Total Number	Seroprevalence
Sample Prevalence	38	827	4.6%
Embryo calves[2]	21	565	3.72%
Conventional calves	17	262	6.58%
Age ≤ 330 days	5	75	6.67%
Age > 330 days	33	754	4.38%
Weight ≤ 386.4 kg	6	50	12.0%
Weight > 386.4 kg	32	777	4.12%

[1] Status determined according to cELISA on samples obtained Day-10.

[2] Pregnancies resultant of embryo transfer in which conception is achieved by artificial insemination within a donor cow and embryos are harvested at Day 7 of pregnancy and transferred to a recipient dam for gestation, parturition, and suckling.

Table 4.4 Comparison of performance and diagnostic variables for purebred Angus yearling bulls receiving an 80-day medicated diet in combination with injectable oxytetracycline on Day 40 (OXYTET) and no injectable antibiotics (CON)

Variable	Treatment		

	OXYTET	CON		
	Mean (95% CI)	**Mean (95% CI)**	**SEM**	***P*-value**
Bulls, head	20	18		
Initial age, days	361 (339, 383)	367 (344, 389)	11.0	0.6921
Bodyweight Day -10, kg	478 (437, 519)	495 (453, 537)	20.5	0.5630
Bodyweight Day 40, kg	529 (487, 570)	546 (503, 589)	21.0	0.5527
Bodyweight Day 80, kg	556 (510, 602)	589 (542, 637)	23.1	0.3083
ADG, Day -10 to Day 40, kg/day	0.994 (0.730, 1.26)	1.01 (0.738, 1.28)	0.133	0.9303
ADG, Day 40 to Day 80, kg/day	0.686 (0.455, 0.917)	1.082 (0.843, 1.320)	0.117	0.0211
Overall ADG, kg/day	0.859 (0.675, 1.04)	1.042 (0.852, 1.23)	0.0928	0.1668
Percent inhibition Day (-)10, %[1]	57.3 (47.9, 66.7)	59.6 (49.9, 69.3)	4.76	0.7314
Percent inhibition Day 40, %	51.2 (31.1, 71.2)	51.6 (31.0, 72.3)	10.11	0.9739
Percent inhibition Day 80, %	54.5 (37.8, 71.2)	54.6 (37.4, 71.9)	8.43	0.9941
Percent inhibition Day 128, %	54.9 (37.4, 72.5)	52.7 (34.5, 70.8)	8.87	0.8552

[1] Percent inhibition = 100 – ([sample OD x 100]/mean OD of negative control sample)

Table 4.5 Comparison of performance and diagnostic variables for purebred Angus yearling bulls on an 80-day medicated diet that remained seropositive[1] for Bovine anaplasmosis at Day 40 (POS) and that converted to seronegative status by Day 40 (NEG)

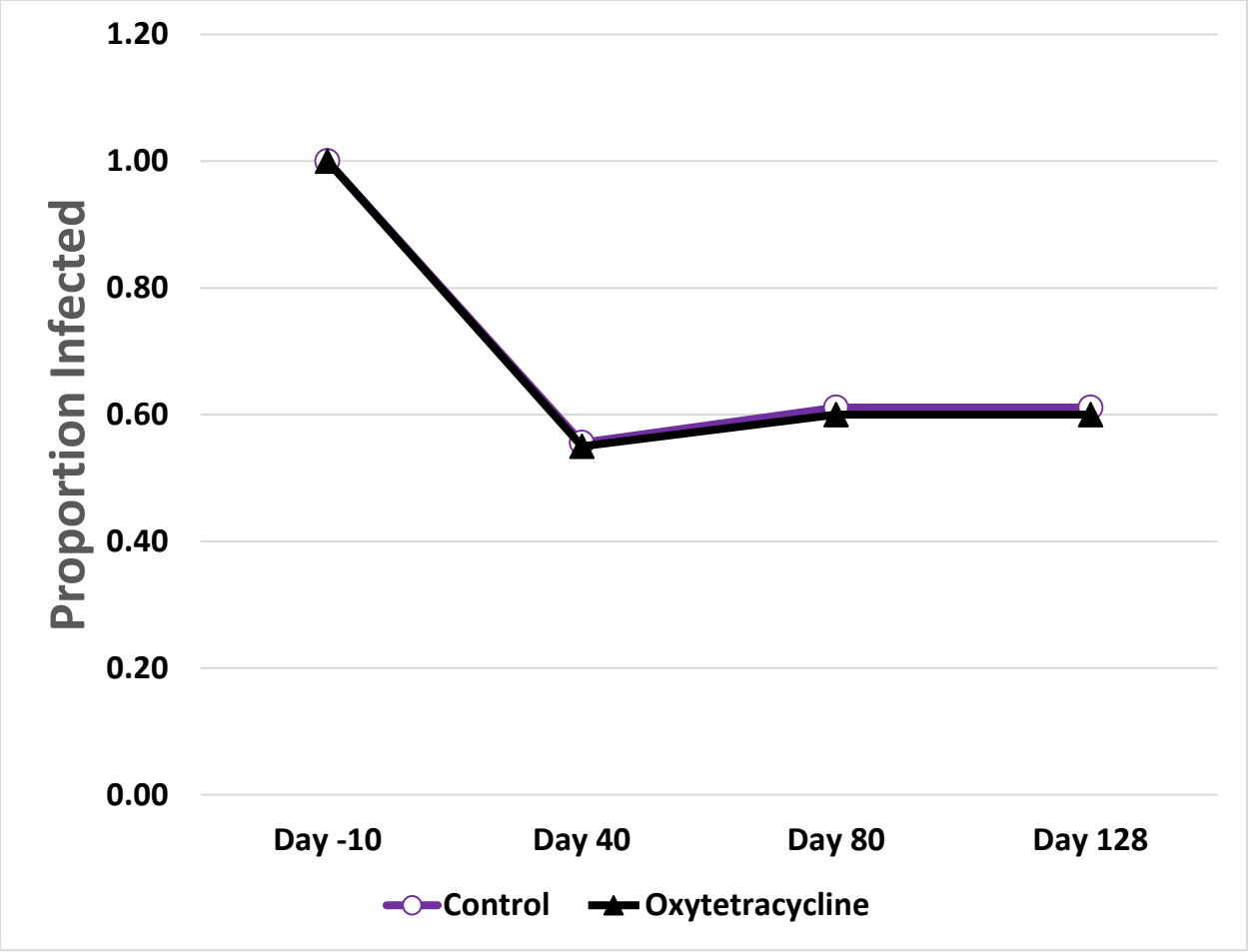
Variable	**Status Change**			
	POS	**NEG**		
	LSMean (95% CI)	**LSMean (95% CI)**	**SEM**	**P-value**

Bulls, head	21	17		
Initial age, days	363 (342,383)	365 (341,389)	11.8	0.8738
Bodyweight Day -10, kg	467 (430, 504)	512 (469, 556)	21.2	0.1173
Bodyweight Day 40, kg	523 (484, 561)	557 (512, 602)	22.2	0.2489
Bodyweight Day 80, kg	1230 (1135, 1325)	1299 (1187, 1411)	54.8	0.3456
ADG, Day -10 to Day 40, kg/day	1.09 (0.847, 1.33)	0.879 (0.593, 1.16)	0.140	0.9303
ADG, Day 40 to Day 80, kg/day	0.908 (0.669, 1.15)	0.836 (0.555, 1.12)	0.137	0.6914
Overall ADG, kg/day	1.01 (0.835, 1.19)	0.86 (0.654, 1.07)	0.1008	0.2656
Percent inhibition Day (-)10, %[2]	72.4 (68.8, 75.9)	39.2 (35.0, 43.3)	2.04	<0.0001
Percent inhibition Day 40, %	81.69 (75.35, 88.0)	9.42 (1.96, 16.9)	3.65	<0.0001
Percent inhibition Day 80, %	80.1 (75.2, 85)	19.2 (13.5, 25)	2.83	<0.0001
Percent inhibition Day 128, %	80.2 (74.3, 86.2)	17.3 (10.3, 24.3)	3.41	<0.0001

[1] Status determined according to cELISA on samples obtained Day-10.
[2] Percent inhibition = 100 – ([sample OD x 100]/mean OD of negative control sample)

Figures

Figure 4.1 Proportion of purebred Angus yearling bulls seropositive for Bovine anaplasmosis grouped by respective treatments at sample time points Day -10, Day 40, Day 80, and Day 128


1.20
1.00
0.80
0.60
0.40
0.20
0.00
Proportion Infected
Day -10
Day 40
Day 80
Day 128
Control
Oxytetracycline