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Original Article

Factors associated with seroprevalence of bovine anaplasmosis in Texas

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ABSTRACT

Bovine anaplasmosis (BA), caused by *Anaplasma marginale,* is an economically important tick-borne disease of cattle in the United States (U.S.) and worldwide. Anecdotally, Veterinary Feed Directive prescriptions in the southeastern U.S. are written mostly for treatment/prevention of BA. However, there are no recent temporal seroprevalence estimates of BA in Texas (TX). Thus, this study was aimed at determining the seroprevalence of and factors associated with BA in TX. Data were obtained from an active slaughter survey (n = 215) performed between August and December 2014 as well as from reviewing Texas A&M Veterinary Medical Diagnostic Laboratories (TVMDLs) records of specimens submitted for BA testing from January 2002 to June 2012 (n = 15,460). Irrespective of the assay used, the overall apparent seroprevalence of BA in TX between 2002 and 2012 was 15.91% (95% CI: 15.34 — 16.50%) and the yearly increase in seroprevalence followed a significant trend (P < .0001). With cELISA, the apparent seroprevalence of BA was 13.49% (95% CI: 9.56 — 18.7%) and 13.02% (95% CI: 9.74 — 17.18%) for the slaughter survey and TVMDLs records between October and December 2011, respectively. Whereas the estimated true seroprevalence for the same period was 12.35% (95% CI: 8.04 — 18.05%) and 12.78% (95% CI: 9.19 — 17.30%), respectively. Factors associated with positive BA results were age, breed, diagnostic assay used, year and quarter of the year the specimens were submitted. The odds of the outcome were 1.5 times as high when cattle were adults (vs juvenile). In comparison to other breeds, the odds of the outcome were 11.57, 7.16, and 2.5 times higher in Hereford, Angus, and mixed breeds, respectively. When compared to 2003, the odds of the diagnosis of BA was approximately 2 times as high in 2010 but 3 times as high in 2002, 2005, and 2011 and approximately 4 times as high in 2006 and 2007. In comparison to the duration from October to December, the odds of the outcome were approximately 1.5 as high from January to March and from July to September durations. Counties with specimen submissions for BA testing had a significantly greater cattle population (p = .0061) and number of cattle farms (p < .001) than counties without specimen submissions. Subsequent prevention and control measures for BA should target these factors and should prioritize on counties with higher cattle population in the eastern part of TX. Furthermore, TVMDLs records appear sufficient for the surveillance of BA in TX.

1. Introduction

Bovine anaplasmosis (BA), caused by the rickettsial hemoparasite *Anaplasma marginale*, is one of the most prevalent tick-transmitted disease of cattle worldwide (Dumler et al., 2001; Kocan et al., 2003; Uilenberg, 1995). Although infectious but non-contagious, BA is a major obstacle to profitable cattle production in the United States (U.S.) as well as in many other countries (Aubry and Geale, 2011; Decaro et al., 2008; Howden et al., 2010; Kocan et al., 2010). *Anaplasma marginale* is commonly transmitted by biological (ticks) or mechanical vectors (biting flies, contaminated needles, and surgical instruments), and less frequently transplacentally (Aubry and Geale, 2011; Kocan

Abbreviations: AAVLD, American Association of Veterinary Laboratory Diagnosticians; BA, Bovine anaplasmosis; CFT, Complement fixation test; cELISA, Competitive Enzyme-linked immunosorbent assays; Se, Sensitivity; Sp, Specificity; TX, State of Texas; US, United States of America; USDA, United States Department of Agriculture; VDL, Veterinary diagnostic laboratory; VFD, Veterinary Feed Directive

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et al., 2010; Radostits and Done, 2007). About 20 species of ticks have been implicated as vectors in the biological transmission of *A. marginale* (Kocan et al., 2010) worldwide. In the U.S., however, interstadial transmission of *A. marginale* has been demonstrated by the 3-host ticks, *Dermacentor andersoni* and *Dermacentor variabilis* (Kocan et al., 2010). Biological vectors are important in disease transmission because *A. marginale* can be maintained and propagated in the vector over an extended period of time, but some strains depend on mechanical transfer, which must be timely since only a fixed amount of agent is transferred (Aubry and Geale, 2011; Kocan et al., 2010; Richey and Palmer, 1990). The incubation period of infection (prepatent period) for *A. marginale* is 28 days on average with a range of 7 to 60 days, mainly due to varying infective dose (Kocan et al., 2010). Once *A. marginale* infects an animal, the organism invades and multiples within erythrocytes, leading infected erythrocytes to undergo extravascular destruction and associated clinical signs. These clinical signs include anemia, icterus, fever, weight loss, abortions, and death (Kocan et al., 2003; Richey and Palmer, 1990).

An introduction of *A. marginale* into a naive herd can result in a 3.6% reduction in calf crop, a 30% increase in cull rate, and a 50% mortality rate in clinically infected adult cattle (Kocan et al., 2010). Cattle surviving BA are important in the epidemiology of the disease. Cattle that recover from acute anaplasmosis, including those treated with recommended doses of tetracycline, maintain a microscopically undetectable parasitemia for life (Aubry and Geale, 2011; Eriks et al., 1989; Kocan et al., 2010; Palmer et al., 2000; Radostits and Done, 2007; Richey and Palmer, 1990). Persistent infection is characterized by cyclic rickettsemia ranging from 10^2 to 10^7 infected erythrocytes per mL of blood that occur at approximately five-week intervals (Eriks et al., 1989; Kuttler and Simpson, 1978; Stewart et al., 1979). Although deaths may still occur, persistent infections usually confer resistance to clinical anaplasmosis (Kocan et al., 2010). Persistently infected cattle exposed to mechanical and/or biological vectors serve as reservoirs of infection to introduce *A. marginale* into populations of naive cattle thereby leading to endemic disease stability (de Echaide et al., 1998; Futse et al., 2003; Reeves and Swift, 1977).

Overall, the cost of a clinical case of BA in the U.S. has been conservatively estimated to exceed $400 per animal (Alderink and Dietrich, 1983; Goodger et al., 1979) with the total cost to the beef industry exceeding $300 million per year. However, the lack of recent information regarding the seroprevalence of BA throughout the U.S. and its economic impact on cattle production make accurate assessment of production losses incurred by the cattle industry in the U.S. difficult, if not impossible to estimate.

Strategies applied to manage BA include diagnostic testing, vector and cattle movement control, reducing iatrogenic (e.g. mechanical through contaminated needles) transmission, and administration of low doses of tetracycline antimicrobials in feed or mineral supplements (Aubry and Geale, 2011). However, effective implementation of control strategies requires knowledge of the local or regional seroprevalence of BA. An estimate of the apparent seroprevalence of BA in TX beef cattle in 2011 was reported as 15.02% (Hairgrove et al., 2014). Although that study provided a very useful information, it is also important to evaluate the temporal seroprevalence of BA in TX cattle. Moreover, additional laboratory results for BA diagnosis in TX in 2011 may support or provide different seroprevalence estimates than was previously reported. Until the Veterinary Feed Directive (VFD) rule in 2017, BA was commonly touted reason for cattle in southeastern U.S. to be administered oral antibiotics for long periods. Since the VFD implementation, we have anecdotal information that most recent antibiotic prescriptions in many southeastern states, including TX, have been for the treatment and/or prevention of BA. Indiscriminate use of antimicrobials in animals is known to increase the prevalence of microorganisms resistant to these antimicrobials (De Briyne et al., 2013). It is therefore important to quantify the presence of and factors affecting BA diagnosis in TX cattle. Estimating the temporal longitudinal seroprevalence of BA in TX is therefore a critical first step to implementing appropriate BA control programs in this state and can be a sentinel for the seroprevalence estimate in the region.

Hence, the objective of this study was to estimate the temporal seroprevalence and risk factors associated with A. *marginale* infections in TX cattle through active purposive screening of beef cows as well as the use of an 11-year previously collected accredited laboratory records. The expected results would provide (1) farmers and policy makers the benchmark tools needed to improve the control of BA in TX, and (2) insights into the reliability of laboratory records in estimating the seroprevalence of BA in TX. Collectively, these efforts would provide opportunities for prevention and management practices targeted to populations of cattle at greater risk of BA.

2. Materials and methods

2.1. Active beef cow screening

Slaughter survey of TX beef cows for BA was performed as previously described (Okafor et al., 2018). Descriptively, based on a population of 4,329,341 beef cows (NASS, 2014), an estimated seroprevalence of 10% (and not < 6%), and a confidence level of 95%, 216 beef cows were required to estimate the seroprevalence of BA in TX beef cows. This sample size was calculated using the Epi Info™ Version 7.0 software (Centers for Disease Control and Prevention, Atlanta, GA, USA). A slaughterhouse that slaughtered a significant portion of beef cattle from TX was purposively selected as a specimen collection site. This slaughterhouse, FLP Food, is located in Augusta, Georgia. Between August and December 2014, blood specimens were collected from cull beef cows at this slaughterhouse. Only one specimen was collected from each sampled cow. For each beef cow, the individual number from a USDA-approved backtag was recorded at the time of specimen collection. Specimens were collected only from cows with backtag identifications beginning with the prefix “74”, indicating TX as the state of last origin; with the first mature incisors erupted, indicating the cow was at least 18 months of age; a phenotype consistent with beef cattle. On specimen collection dates, blood specimens were collected from all beef cows that met the above criteria. During exsanguination, blood was collected (~8 mLs) from each cow in a new blood collection tube (BD Vacutainer Serum Separator; 8.5 mL). All blood specimens were transported in ice-pack containers and tested with cELISA, using the Anaplasma Antibody Test Kit (VMRD, Pullman, WA). In accordance with commercial testing guidelines, all specimens having a ≥ 30% inhibition were reported as serologically positive.

2.2. Laboratory records evaluation

The computer records of all BA diagnosis performed between January 2002 and July 2012 were obtained from two American Association of Veterinary Laboratory Diagnosticians (AAVLD) accredited Veterinary diagnostic laboratories (VDLs) in Texas (TX). The participating laboratories were the Texas A&M Veterinary Medical Diagnostic Laboratory in College Station, TX (TVMDL-College Station) and the other in Amarillo, TX (TVMDL-Amarillo). Obtained records included date of specimen submission, geographic information (state, county, city, and/or zip code associated with the submission), breed and/or type of cattle, age, the diagnostic assay used, and the test result. Cattle breeds with < 500 animals were collectively categorized to as ‘other’. For most cattle, age in months were captured in addition to further categorical description of the animal as either adult or juvenile. According to the records, any animal whose age was < 24 months was classified as juvenile and anyone whose age was ≥24 was classified as adult. The BA assays used by these VLDs were card test, complement fixation test (CFT), competitive enzyme linked immunosorbent assay (cELISA), and quantitative polymerase chain reaction (qPCR). To facilitate analysis, all submissions without definite positive or negative