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Short Communication

Seroprevalence of bovine Anaplasmosis in Georgia

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ABSTRACT

Anecdotally, Veterinary Feed Directive prescriptions in many states in the southeastern United States (U.S.) are written most often for treatment and prevention of bovine anaplasmosis (BA). This tick-borne disease of cattle caused by *Anaplasma marginale* remains an economically important disease in U.S. However, there are no prevalence estimates of this disease in Georgia (GA). Thus, this study was aimed at determining the seroprevalence of BA in GA. In an active cull beef cow screening for BA, 293 beef cows were sampled from one cattle auction barn and one slaughterhouse between May 2013 and September 2014. These cows originated from 6 of 159 counties in GA. The top 3 counties sampled were Gordon (241 samples), Carroll (25 samples), and Emanuel (12 samples). Of the 293 sampled beef cows, 13 were positive and 280 were negative for BA. Hence, with competitive ELISA, the overall observed apparent seroprevalence of BA in GA was 4.44% (95% CI: 2.61–7.44%) while the estimated true seroprevalence was 2.62% (95% CI: 5.2–5.87%). The top 2 prevalent counties were Carroll and Gordon with apparent seroprevalence of 8% (95% CI: 2.22–24.97) and 4.78% (95% CI: 2.69–8.36), respectively and estimated true seroprevalence of 6.45% (95% CI: 0–25.37) and 2.99% (95% CI: 0.54–6.89), respectively. Although not significant, counties with specimen submissions for BA testing had a greater cattle population and number of cattle farms than counties without specimen submissions. Nevertheless, future prevention and control measures for BA should out of caution target counties with ≥5000 total cattle heads.

1. Introduction

The lack of recent information regarding the prevalence of bovine anaplasmosis (BA) throughout the United States (U.S.) make accurate assessment of production losses incurred by the cattle industry in the U.S. difficult, if not impossible. Bovine anaplasmosis, caused by *Anaplasma marginale*, is one of the most prevalent tick-transmitted disease of cattle worldwide (Dumler et al., 2001; Kocan et al., 2003). This infectious but non-contagious disease remains a major obstacle to profitable cattle production in the U.S. (Decaro et al., 2008; Howden et al., 2010). The introduction of *A. marginale* into a naive herd can result in a reduced in calf crop, an increased cull rate, and a mortality rate of ≤50% in clinically infected adult cattle (Kocan et al., 2010). Historically the cost of a clinical case of BA in the U.S. has been 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.

Infection is transmitted by biological (ticks; *Dermacentor andersoni* and *Dermacentor variabilis* in the U.S.) or mechanical vectors (biting flies), fomites (e.g. contaminated needles), and less frequently through transplacental transmission (Aubry and Geale, 2011; 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, 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). The incubation period of infection (prepatent period) for *A. marginale* varies with the infective dose with an average of 28 days (Kocan et al., 2010). Once an animal is

Abbreviations: BA, Bovine anaplasmosis; cELISA, Competitive Enzyme-linked immunosorbent assays; GA, State of Georgia, USA; Se, Sensitivity; Sp, Specificity; U.S., United States of America; USDA, United States Department of Agriculture; VFD, Veterinary Feed Directive

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infected, *A. marginale* invades and multiples within erythrocytes, which undergo extravascular destruction and associated clinical signs. Clinical signs associated with BA include anemia, icterus, fever, weight loss, abortions, and death (Kocan et al., 2010). Cattle surviving BA maintain a bacteremia for life (Aubry and Geale, 2011; Palmer et al., 2000). Although deaths may still occur, persistent infections usually confer resistance to clinical anaplasmosis (Kocan et al., 2010). Persistently infected cattle exposed to 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).

Strategies applied to manage BA include diagnostics, control of vector and cattle movement, reducing iatrogenic transmission, and administration of low doses of tetracycline antimicrobials in feed or mineral supplements (Aubry and Geale, 2011). BA is a commonly touted reason for southeastern U.S. cattle to be administered oral antibiotics for long periods. Indiscriminate use of antimicrobials in animals is known to increase the prevalence of microorganisms resistant to these antimicrobials (De Briyne et al., 2013). Therefore, there is growing concern about the prevalence, economic impact, and effects on judicious use of antibiotics of BA in cattle in the southeastern U.S. Estimating the seroprevalence of BA in GA is therefore a critical first step to implementing appropriate BA control programs in this state and can be a sentinel for the prevalence estimate in the region. The last reported prevalence of BA in the greater southern U.S. region occurred in the 1970's, ranged from 2% to 24%, and did not include data from GA (McCallon, 1973).

Thus, the objective of this study was to estimate the seroprevalence of *A. marginale* infections in GA beef cattle through active purposive screening of cull beef cows. The expected results would provide farmers and policy makers the information needed to improve the control of BA in GA. 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 cull beef cow screening

Required sample size for the seroprevalence estimate was determined as described previously (Okafor et al., 2018a,b). Based on an estimated prevalence of 5% (and not < 2.5%), a confidence level of 95%, and a population of 469,942 beef cows from the 2012 census of the National Agricultural Statistical Service (NASS, 2014), 292 beef cows were required to estimate the prevalence of *A. marginale* in GA 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 cattle auction barn and a slaughterhouse that sold and slaughtered, respectively, a significant portion of beef cattle from GA were purposively selected as specimen collection sites. The cattle auction barn, Calhoun Stockyard, and the slaughterhouse, FPL Food, were located in Calhoun, Georgia and Augusta, Georgia, respectively. Between May to August 2013 (Calhoun Stockyard) and September 2014 (FPL Food), blood specimens were collected from cull beef cows. Hence, at the stockyard/auction barn, only beef cows that were intended for slaughter were sampled. Specimens were collected only from cows with a USDA-approved backtag identifications beginning with the prefix “57”, indicating GA 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. Only one specimen was collected per animal and all specimens in each location were collected by the same individuals (one collected all the specimens at the slaughterhouse and another collected all the specimens at the auction barn). At the slaughterhouse, blood was collected (~8 mLs) from each cow in a new blood collection tube (BD Vacutainer Serum Separator; 8.5 mL) during exsanguination. At the cattle auction barn, blood was also collected (~8 mLs) from each cow's coccygeal vein with a new needle (BD Vacutainer Single-sample Needle; 18 gauge; 3.8 cm) and a new blood collection tube. Since the collection at the auction barn was from live animals, the University of Tennessee Animal Care and Use Committee approved the protocol for the specimen collection (protocol 2175). All blood specimens were transported in ice-pack containers and tested with competitive ELISA (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. Analysis

In estimating the true prevalence of BA, previously described sensitivity (Se) and specificity (Sp) results of cELISA was used (Aubry and Geale, 2011; Coetzee et al., 2007). The Se and Sp results for cELISA were 95.0% and 98.0%. True prevalence estimates were calculated as described previously (Reiczigel et al., 2010; Rogan and Gladen, 1978). Furthermore, Wilson's confidence intervals were calculated on the assumption that Se and Sp were known exactly as described previously (Reiczigel et al., 2010). Cattle population data for each county in GA were obtained from the 2012 census (NASS, 2014) to determine if cattle population and farm type (beef or dairy and size of cattle operations) differed for counties without specimen submissions, with specimen submissions having only negative results, and with specimen submissions having both negative and positive results. Additionally, obtained results were presented visually as a choropleth map using ArcGIS 10.5 (ESRI, Redlands, CA).

3. Results

From the slaughterhouse and stockyard, 293 cull beef cows were sampled and 13 were positive whereas 280 were negative for BA (Fig. 1). Hence the overall observed apparent seroprevalence of BA in GA was 4.44% (95% CI: 2.61–7.44%) while the estimated true seroprevalence was 2.62% (95% CI: 5.2–5.87%). These cows originated from 6 of 159 counties in GA. This county information corresponds to the stockyard where the animal received its backtag identification and may not necessarily correspond to the county of residence before sale and subsequent slaughter. There were approximately 45 stockyards in 35 counties approved to sell cattle in GA during the survey, and these cull beef cows originated from 6 (13.33%) of those 45 stockyards and 6 (17.14%) of those 35 counties. Of those 6 counties, 2 counties had 266 specimens that yielded 13 positive and 253 negative test results while 4 counties had only negative test results (27 samples); greater samples were obtained from Gordon (241 samples), Carroll (25 samples), and Emanuel (12 samples) (Table 1). Per county, the number of tested cattle ranged from 1 to 241 (median = 11; mean = 49). The top 2 prevalent counties were Carroll and Gordon with apparent seroprevalence of 8% (95% CI: 2.22–24.97) and 4.78% (95% CI: 2.69–8.36), respectively and estimated true seroprevalence of 6.45% (95% CI: 0–25.37) and 2.99% (95% CI: 0.54–6.89), respectively. The median cattle population and number of cattle farms among counties whose cattle were sampled for BA was 7868 and 133, respectively; whereas the median cattle population and number of cattle farms among counties whose cattle were not sampled for BA was 4501 and 82, respectively (Table 2). Although the total cattle population and the number of cattle farms was higher among counties whose cattle were sampled for BA when compared to those that were not, this difference was not significant for total cattle population ($p = .0607$) and the number of cattle farms ($p = .1468$).

4. Discussion

The study reported here describes for the first time estimates of the seroprevalence of BA in GA. The apparent and true seroprevalence estimates were 4.44% and 2.62%, respectively. These estimates were obtained from cull beef cows sampled in the summer and tested using

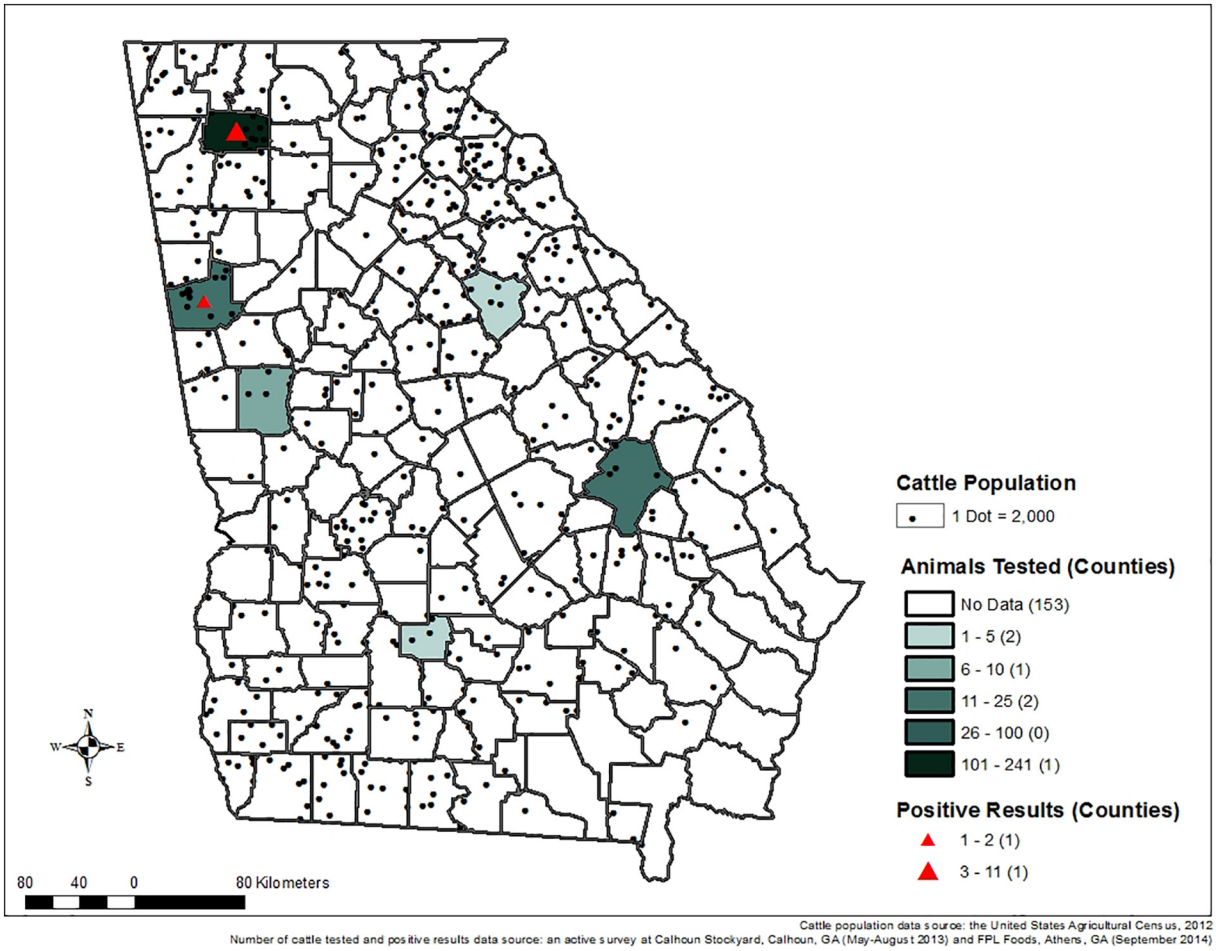

Fig. 1. Choropleth map of cattle population density per county in Georgia, number of beef cows tested, and positive results and their distribution based on prospective surveillance data for bovine anaplasmosis, 2013–2014.

cELISA. This sampling frame used in the evaluation was positively associated with diagnosis of BA in other states in southern U.S. (Okafor et al., 2018a,b). Specifically, in comparison to dairy cattle and other months of the year, beef cattle and sampling in the summer months were shown to increase the likelihood of a positive BA results in Kentucky (Okafor et al., 2018b) and in Texas (Okafor et al., 2018a). In both studies, cELISA was associated with a positive diagnosis of BA in comparison to complement fixation test. Although cELISA provides higher seroprevalence estimates of BA when compared to complement fixation test, it remains an optimal assay for estimating the seroprevalence of *A. marginale* in U.S. cattle at the moment (Okafor et al., 2018a,b). In spite of this sampling advantage, seroprevalence estimates in GA appear lower than other southern states such as Kentucky (Okafor et al., 2018b) and Texas (Hairgrove et al., 2015; Hairgrove et al., 2014; Okafor et al., 2018a). Comparably, GA has fewer cattle than states such as Texas and Kentucky and such difference in cattle demography could have contributed to the comparable lower seroprevalence estimates of BA observed in GA.

Following the introduction of a carrier animal from an endemic area, previously naive cattle in non-endemic areas may become infected with *A. marginale* after a blood meal from an infected vector (Smith et al., 1989). In warmer months, most animals spend more hours of the day in the pasture when ticks have high reproducibility and mobility (Alderink and Dietrich, 1983). Hence, clinical outbreaks of BA occur most frequently during this season because of optimal opportunities for exposure between naive animals and infected ticks (Alderink and Dietrich, 1983). Rarely, iatrogenic *A. marginale* infection associated with contaminated surgical equipment or hypodermic needles may give rise to occurrence of clinical cases outside the normal vector season (Smith et al., 1989). Although all cattle are susceptible to infection by

Table 1
Apparent and estimated true seroprevalence of bovine anaplasmosis in Georgia counties estimated with stockyard and slaughterhouse surveys, between May to August 2013 and September 2014, respectively.

County	Total cattle population	Number of beef farms	Number of dairy farms	Number of beef cows screened for Anaplasmosis by cELISA (no. Positive)	Apparent prevalence for Anaplasmosis by cELISA (95% CI)	Estimated true prevalence for Anaplasmosis by cELISA (95% CI)
Carroll	20,822	471	11	25 (2)	8 (2.22–24.97)	6.45 (0–25.37)
Gordon	18,583	356	2	230 (11)	4.78 (2.69–8.36)	2.99 (0.54–6.89)
Emanuel	7172	131	1	12 (0)	< 0 (0–25.25)	< 0 (0–23.94)
Meriwether	7039	109	3	9 (0)	< 0 (0–29.91)	< 0 (0–32.62)
Greene	8563	85	11	5 (0)	< 0 (0–43.45)	< 0 (0–51.61)
Turner	4819	89	4	1 (0)	< 0	< 0

Table 2
Distribution of cattle and farm demographics between counties associated with cattle tested for bovine anaplasmosis in Georgia and those that were not during stockyard and slaughter surveillance between May 2013 and September 2014.

Counties associated with cattle tested for bovine anaplasmosis (n = 6)

Variable	Minimum	Maximum	Mean	Lower 95% CL	Upper 95% CL	Standard deviation	Median
Cattle population	4819	20,822	11,166	4075	18,258	6757	7868
Number of farms	92	529	233	40	427	185	133
Beef farms	85	471	207	34	380	165	120
Dairy farms	1	11	5	1	10	5	4

Counties not associated with cattle tested for bovine anaplasmosis (n = 153)

Variable	Minimum	Maximum	Mean	Lower 95% CL	Upper 95% CL	Standard deviation	Median
Cattle population	151	24,882	6445	5481	7409	5974	4501
Number of farms	2	500	104	90	118	87	82
Beef farms	1	474	91	79	104	79	72
Dairy farms	1	28	4	3	4	4	2

A. marginale, seroprevalence of BA have been demonstrated to be significantly higher in beef breeds in comparisons to dairy breeds (Kocan et al., 2003; Okafor et al., 2018a; Okafor et al., 2018b). Breeds and/or type of cattle that spend greater time in pasture than in shelter/barn (e.g. beef compared to dairy cattle) may be at increased risk due to higher likelihood of exposure to transmission vectors (Haskell et al., 2006; Simon et al., 2016). Routinely, beef cows are retained longer than beef bulls because of the economics associated with production of calves. When beef cows are culled, it is usually because they are aged (> 10 years of age), have bad teeth, are infertile, or have some morbidity (NAHMS, 2010). Furthermore, BA manifestation and detection appear more in cattle ≥24 months of age in comparison to those < 24 months (Aubry and Geale, 2011; Coetzee et al., 2005; Okafor et al., 2018a; Okafor et al., 2018b). Intuitively, with increasing age of cattle the likelihood being parasitized by vectors or infected through iatrogenicity also increases. Because adult cull beef cows were used in this seroprevalence estimation and the sampling occurred in the summer using cELISA, the true seroprevalence of the entire cattle population in GA should be ≤2.5% given that the present study sampled the population at the greatest odds of disease and at a time that diagnosis was mostly higher.

Seroprevalence of BA has been shown to be higher in counties with relatively higher cattle population (Okafor et al., 2018a; Okafor et al., 2018b). Seroprevalence was significantly higher in counties with median total cattle population of 21,000 compared to counties with median total cattle population of 6300 in Kentucky (Okafor et al., 2018b) and in counties with median total cattle population of 38,000 compared to counties with median total cattle population of 14,000 in Texas (Okafor et al., 2018a). However, in the present study, counties with specimen submissions for BA testing (median total cattle population of 7868) did not have a significant greater cattle population than counties without specimen submissions (median total cattle population of 4501). This could be because sampled cattle originated from a small proportion of counties in the state. Furthermore, these county seroprevalence estimates may not necessarily reflect the true estimate in each county of residence of cattle because in actuality the county information corresponds to the stockyard where the animal received its backtag identification. Since, there is no other metric to measure the cattle's current county of residence, we utilized the identification county information available. So, if the cattle are traded to another county after the backtag identification, the estimate could be erroneous. Any such misclassification of cattle by county would likely be the same for counties irrespective of their total cattle population (i.e., non-differential misclassification). Hence, such bias would be toward the null and would not have favored any specific county in this study. The effect of herd size on seroprevalence of BA were not evaluated in the present study but there appears to be conflicting reports on this subject. In Texas, large cattle herds appeared to sustain BA infection more persistently than smaller herds, as the percent of herds reporting clinical cases increased as herd size increased (Alderink and Dietrich, 1983). In Louisiana, however, the seroprevalence of cattle to BA was independent of herd size (Hugh-Jones et al., 1988). Although total cattle population was not found to be associated with BA, prevention and control measures for BA in GA should cautiously be targeted at counties with ≥5000 total cattle heads because of the difference in median total cattle population between counties with specimen submissions for BA testing (n = 7868) and those counties without specimen submissions (n = 4501).

5. Conclusion

The estimated true seroprevalence of BA in GA was 2.62% (95% CI: 5.2–5.87%). This estimate appears lower than those of other southern U.S. states with available seroprevalence estimates. Current control measures for BA should be upheld and future measures should cautiously target counties with ≥5000 total cattle heads.

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Declaration of conflicting interests

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Ethical statement

Prior to the onset of this study, The University of Tennessee Knoxville Institutional Animal Care and Use Committee was queried regarding the need for an Institutional Animal Care and Use Protocol. In the slaughterhouse survey, blood samples were collected during exsanguination, after cows were humanely stunned with a penetrating captive bolt. As our study did not interfere with the regular humane treatment of animals during slaughter at a USDA inspected plant, an

approved protocol was not required, per direction of the Committee. However, since the specimen collection at the auction barn was from live animals, the University of Tennessee Animal Care and Use Committee approved the protocol for the specimen collection (protocol 2175).

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