Seroprevalence and molecular detection of *Anaplasma marginale* infected beef herds in Georgia, USA

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Abstract
The objective of the study was to conduct and compare seroprevalence and molecular detection of *Anaplasma marginale*-infected beef herds in Georgia, and to identify herd risk factors associated with *A. marginale*-positive herds. Herd information from the beef operations was collected from the sampled herds through a questionnaire to determine practices that may affect the risk of herd infections with *A. marginale*. Blood samples were collected from 1,059 adult beef cattle (≥ 2 years) from 33 herds. Overall, 8.12% of cattle and 42% of herds were cELISA antibody-positive. Seventy-seven percent of plasma samples from a subset of corresponding seropositive samples (n = 73) were PCR positive. All of the 406 seronegative samples were negative by PCR. There was almost perfect agreement by Cohen's kappa statistics between PCR and cELISA (k = 0.85). Survey response rate was 100%. Surprisingly, 27% of the producer respondents had not heard of anaplasmosis. Survey also revealed that many producers had management procedures at their operations (dehorning, castration, tattooing, others) that could potentially cause mechanical transmission of *A. marginale*. About 55% of respondents did not always disinfect tools between animals and 88% of the operations used the same needle to inject more than one animal. Over 80% of the producer respondents had brought in new cattle onto their operations in the last 3 years from the time of this study. Given the 8% seroprevalence in Georgia beef cattle and the reported survey findings from this study, educational programs on effective preventive management practices to control bovine anaplasmosis is warranted.

Key words: *Anaplasma marginale*, anaplasmosis, beef cattle, Georgia, seroprevalence

Introduction
Bovine anaplasmosis (BA) is an infectious, non-contagious blood borne disease that affects cattle worldwide[1] and one of the most prevalent tickborne diseases diagnosed around the world.[2] It is endemic in the Southeast and midwestern United States and has been reported in all the states in the U.S. except Hawaii. In Georgia, the incidence has been increasing since 2009 according to veterinary diagnostic laboratory data.[3] It is estimated that the economic burden of anaplasmosis to the beef industry is more than $300 million per year.[4] The economic losses of anaplasmosis include decreased production and fertility, mortality, abortion and clinical treatment expense. In the U.S., the cost of a clinical case of anaplasmosis is conservatively estimated at > $400 per animal.[5] In one Iowa dairy herd study, seropositive dairy cows produced significantly less milk than did seronegative herdmates.[6] If cattle recover from the disease, they become persistently infected carriers for life, which confers resistance to clinical disease, but serves as a significant reservoir of *Anaplasma marginale* (*A. marginale*) to expose naïve cattle.[7]

In North America, BA is caused by the rickettsial organism *A. marginale*, which lives in the red blood cells (RBC) of cattle.[7] Anaplasmosis is not contagious but requires a vector to be transmitted from one animal to another. The primary method of transmission is by ticks (biological host) but transmission is strain-dependent. Some strains of *A. marginale* are not transmissible by ticks[8] and are more likely transmitted mechanically by blood feeding flies.[9] There are several species of ticks that can spread the disease including *Rhipicephalus* (*Boophilus*) (southern cattle ticks) and *Dermacentor* spp. including *D. andersoni* and *D. variabilis*. *A. marginale* may be transmitted transstadially throughout the tick's life cycle. Mechanical vectors such as blood feeding flies and blood-contaminated fomites such as needles, ear-tagging instruments, castrating knives, dehorning tools and implant guns can also transfer infected erythrocytes to naïve cattle.

Cattle of all ages are susceptible to infection, but severity of disease increases with age of the animal. Calves less than 1 year of age may show no clinical signs following infection; animals 1 to 2 years of age may display mild illness; while animals over 2 years of age often experience the more severe clinical disease, and animals over 3 years of age may experience 30 to 50% mortality.[2,10] Once within the blood stream,

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A. marginale invades erythrocytes and replicates for about 15 to 30 days (incubation ranges from 7 to 60 days depending on exposure and strain).[11] Clinical signs vary and commonly include fever, loss of appetite, pale or yellow mucous membranes (caused by anemia), poor body condition, abortion, weakness and labored breathing. Animals may experience abnormal behavioral signs such as increased aggression, excitability or staggering due to effects of hypoxia. Abortions can occur when individuals are infected during late-stage gestation and should be considered in herd abortion cases. Also, one study has demonstrated that 16% of pregnant carrier cows passed anaplasmosis in utero resulting in persistently infected offspring.[12] Regardless of clinical signs, once animals become infected, they are carriers for life and are a source of transmission to naïve members of the herd.[10] Outbreaks of disease occur either when susceptible, unexposed animals are exposed to infected vectors, or when carriers (like a bull or replacement cows and heifers) are brought onto the farm. It is recommended to test all purchased cattle, including new yearling bulls, for anaplasmosis before commingling with the herd.

Anaplasmosis is usually diagnosed by clinical signs and physical examination findings. Laboratory methods for diagnosing *A. marginale* in cattle include stained blood smear for direct microscopic detection of parasites in RBCs, competitive enzyme-linked immunosorbent assay (cELISA) for detection of antibodies for *A. marginale*, and polymerase chain reaction (PCR) for detection of *A. marginale* DNA.[1] Blood smears are a quick, inexpensive method to diagnose *A. marginale* infection in clinically diseased cattle, but the sensitivity is very low. The cELISA detects antibodies for *A. marginale* and is relatively inexpensive, so it is useful for seroprevalence screening of herds for *A. marginale*, but it may give false negative results early in infection prior to development of an antibody response. Polymerase chain reaction is the most sensitive of the tests and useful in detecting small amounts of *A. marginale* DNA in clinically normal cattle, but it is more expensive than blood smear or cELISA. Additionally, persistently infected cattle do not always have detectable amounts of organisms in their blood because of the cyclical nature of the infection with the organism.[7]

Historically, treatment of anaplasmosis consisted of injectable oxytetracycline (OTC). However, injectable OTC has been shown to be ineffective in clearing *A. marginale* infections in carrier cattle.[13] On the other hand, the label dose of oral chlortetracycline (CTC) was shown to effectively chemosterilize persistently infected calves.[14] Free choice oral CTC is approved for control of active infection of anaplasmosis at 0.5 to 2 mg/lb (1.1 to 4.4 mg/kg) body weight per day. Control of active *A. marginale* infection is the only label approved free choice oral CTC indication in cattle. Therefore, in order to write a valid Veterinary Feed Directive (VFD) to feed CTC, veterinarians must have previously diagnosed *A. marginale* in the herd, have seropositive cattle or be aware of herds that have active infections or have had *A. marginale* diagnosed in the area.

Because cattle serve as an important reservoir for *A. marginale* and cattle movement can be a significant source of transmission from infected herds in endemic areas to negative herds and potentially spread to non-endemic areas, information on infection prevalence is beneficial in the development of prevention, treatment and control strategies in beef herds in Georgia. Additionally, prevalence information may assist veterinarians and extension personnel to educate farmers in Georgia and the need for control measures to prevent introduction into their herds.

Materials and methods

Sample collection

Beef herds in Georgia were enrolled by advertising the surveillance project in the *Georgia Cattleman* magazine and using the Georgia Cattlemen's Association eBlast communication. Producers who responded and volunteered their herds for the study were asked to complete a survey about management practices and procedures such as vaccinations and needle changing practices and external parasite control. Owners were also asked to sign an informed consent and waiver of liability as required by the Clinical Review Committee. The project was approved by the University of Georgia, College of Veterinary Medicine Clinical Review Committee (CR-537).

Paired blood samples were collected via coccygeal venipuncture using 10 cc vacutainer tubes (one red top and one purple top containing ethylenediaminetetraacetic acid [EDTA]) from each cow sampled. Tubes were kept in a cooler and returned to the Tifton Veterinary Diagnostic and Investigational Laboratory (TVDIL) or Athens Veterinary Diagnostic Laboratory (AVDL) for harvesting serum or plasma the same day or shipped on icepacks to the laboratory overnight.

Sampling design

Georgia beef cattle were enrolled between April 2018 and June 2019 using a 2-stage convenience sampling design. Cow-calf producers who were members of the Georgia Cattlemen's Association were solicited to participate in the study, and within participating herds, a convenience sample of cattle > 2 years of age was selected for testing. The number of cows selected in each herd was dependent on herd size; 30 adult cattle were selected by convenience in herds with 30 or more cows and all adult cattle were selected in herds with fewer than 30 cows. Producers were given the option of testing more than 30 cattle if they were willing to pay the additional laboratory fees. A sample size of 30 cattle per farm was determined to provide a 95% probability of detecting at least 1 infected cow in herds with a minimum infection prevalence of 10%. Likewise, a minimum sample size of 30 herds was determined to provide a 95% probability of detecting at least 1 infected herd, assuming a minimum herd-level *A. marginale* infection prevalence of 10%.

Anaplasma cELISA

A commercial cELISA was used in our study for the detection of antibodies specific for *Anaplasma* in the bovine serum samples per the instructions provided by the manufacturer in the kit insert.[a] Multiple lots of cELISA kits were verified for this study by including test-positive and test-negative controls as per the manufacturer's instructions. The optical density (OD) of each well was measured by use of an ELISA plate reader at a wavelength of 620, 630 or 650 nm. Percentage inhibition (PI) of each sample was calculated by use of the following equation: % I = 100 (1- [Sample OD ÷ Negative Control OD]). Samples that had inhibition of < 30% were recorded as negative results, whereas samples that had inhibition of ≥ 30% were recorded as positive results.

Nucleic acid purification

Nucleic acid was purified from 200 µl whole blood (EDTA) samples by an automated bead-based extraction method, MagMax Pathogen RNA/DNA kit,[b] using the Qiagen BioSprint 96 magnetic particle processor[c] following the manufacturer's

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instructions. Two µl of Xeno internal positive control DNA[d] (10,000 copies/µl) is spiked as an internal positive control for the DNA purification process and helps monitor for the presence of PCR inhibitors. The extracted DNA samples were stored at 5°F (-15°C) to -13°F (-25°C) until use.

Anaplasma marginale real-time PCR

A real-time PCR assay was used in our study for the diagnosis of *A. marginale* infection of cattle. This assay was highly specific, as it did not cause cross-reactions with other *Anaplasma* species of ruminants, including the closely related *Anaplasma centrale*, or other haemoparasites of ruminants (*Anaplasma bovis, Anaplasma ovis, Anaplasma phagocytophilum, Babesia bovis, Babesia bigemina, Theileria annulata* and *Theileria buffeli*).

The PCR primers and probe[e] targeted a 95 bp portion of the *msp1b* gene.[15] The sequences for the forward primer, reverse primer and probe are as follows: AM-For 5'- TTGGCAAGGCAGCAGCTT-3'; AM-Rev 5'-TTCCGCGAGCATGTGCAT-3'; AM-probe 5'- 6FAM-TCGGTCTAACATCTCCAGGCTTTCAT-BHQ1- 3'.

Each 25 µL PCR reaction was composed of 10 µL TaqMan universal PCR Master Mix,[f] 0.3 µM of *A. marginale* forward primer, 0.3 µM of *A. marginale* reverse primer, 0.2 µM of *A. marginale* probe, 5 µL of nuclease-free water, 1 µL of VetMAX Xeno IPC-VIC assay,[g] and 5 µL of purified nucleic acid. An *A. marginale* target positive control was included in addition to the xeno internal positive control (Xeno IPC-VIC assay) for each PCR run. The thermocycling program used on the ABI 7500 Real-time PCR instrument[h] consisted of a 122°F (50°C) incubation for 2 minutes, 203°F (95°C) incubation for 10 minutes, and followed by 45 cycles of 15 seconds at 203°F (95°C) and 1 min at 140°F (60°C). After the PCR, result analysis was performed with auto-baseline and auto-threshold settings. All samples with a cycle threshold (Ct) value ≤ 40 were recorded as positive results and those with Ct values > 40 or undetermined, were recorded as negative results in accordance with the interpretation criteria described by Carelli et al.[15]

Statistical methods

For samples that were tested using both the cELISA test and the real time PCR test, the marginal percentages of positive results were compared using an exact McNemar's test. The agreement between the 2 tests was evaluated using Cohen's kappa statistic with qualitative interpretation following the scale recommended by Landis and Koch.[16] All statistical testing assumed a 2-sided alternative hypothesis, and values of $P < 0.05$ were considered statistically significant.

Data were screened for errors using range and logic checks. Because the sampling fractions varied across herds, a weighted analysis was performed to reduce potential biases in the prevalence estimation. Initial sampling weights for individual cows were calculated as (N⁄n) × (M⁄m), where N is the number of herds in the population, n is the number of herds in the study sample, M is the total number of cows in the herd, and m is the number of cows that were sampled from the herd. Sampling weights were then adjusted by post-stratification on herd size category (i.e., 1 to 49 cows, 50 to 199 cows and 200 or more cows) so that they would sum to the population sizes within each stratum based on Georgia beef cattle population estimates reported in the United States Department of Agriculture's National Agriculture Statistics Service 2017 Census of Agriculture. A commercial software package[i] with algorithms designed for the analysis of complex survey data was used to obtain population-based estimates while adjusting for sampling weights, herd-level clustering and stratification by herd size.

The apparent prevalence of *A. marginale* infection was estimated using the survey proportion procedure in Stata with 95% confidence intervals estimated using the logit transformation method.[17] The true prevalence of *A. marginale* infection was estimated using the formula (AP + Sp-1)/(Se + Sp-1), where AP is the apparent prevalence of infection, Sp is the specificity of the cELISA test, and Se is the sensitivity of the cELISA test.[18] The sensitivity and specificity of the cELISA assay were assumed to be 95% and 98%, respectively, based on previously reported estimates using a cut-off point of 30% inhibition.[7,19]

Univariable and multivariable associations between herd characteristics and *Anaplasma* seroprevalence were evaluated using survey logistic regression while adjusting for sampling weights, herd-level clustering, and stratification by herd size. Variables having $P < 0.1$ in the univariable analysis were considered eligible for inclusion in the multivariable model. Model selection for the multivariable analysis was performed using a manual backward elimination procedure. Variables were eliminated from the multivariable model in a stepwise manner based on their level of significance until only variables having $P < 0.05$ remained. Due to the exploratory nature of the analysis, 2-way interactions between the predictor variables were not evaluated. Goodness of fit for the final multivariable model was evaluated using the Svylogitgof procedure in Stata, which is an analog of the Hosmer-Lemeshow goodness of fit test designed for use with complex survey data.[20]

Results

Prevalence estimation

Anaplasma antibody testing was performed on sera from 1,059 adult cattle in 33 beef herds. Herd sizes ranged from 12 to 700 cows, with a median of 68 cows. The geographic distribution of herds is illustrated in Figure 1. Positive cELISA results were recorded for 86 (8.1%) cattle and 14 (42.4%) herds. Within herds that had one or more positive cELISA results, the seroprevalence ranged from 5% to 85% with a median of 14%. After adjusting for the 2-stage sampling design, the apparent prevalence of *Anaplasma* infection in Georgia beef cattle was estimated as 8.4% (95% CI: 3.9%, 17.2%), and the true prevalence was estimated as 6.9% (95% CI: 2.0%, 16.3%).

Agreement between PCR and the cELISA test

Real-time PCR was performed on a subset of samples for comparison with results of the cELISA antibody assay (Table 1). In paired testing, the cELISA identified a significantly higher percentage of positive results than did the PCR test ($P < 0.001$). The PCR test yielded positive results for 56 (76.7%) of 73 samples with a positive cELISA result, and for none (0.0%) of 406 samples with a negative cELISA result. Despite the significant difference in the proportions of positive results, the kappa statistic indicated that there was almost perfect agreement between the two tests (kappa = 0.85; 95% CI: 0.78, 0.92).

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Figure 1: Distribution of 33 Georgia beef herds that participated in an *Anaplasma* seroprevalence study by county. Red dots represent herds with one or more positive cELISA results and black dots represent herds with no positive cELISA results. Dots were placed randomly within counties to preserve the anonymity of participating producers.

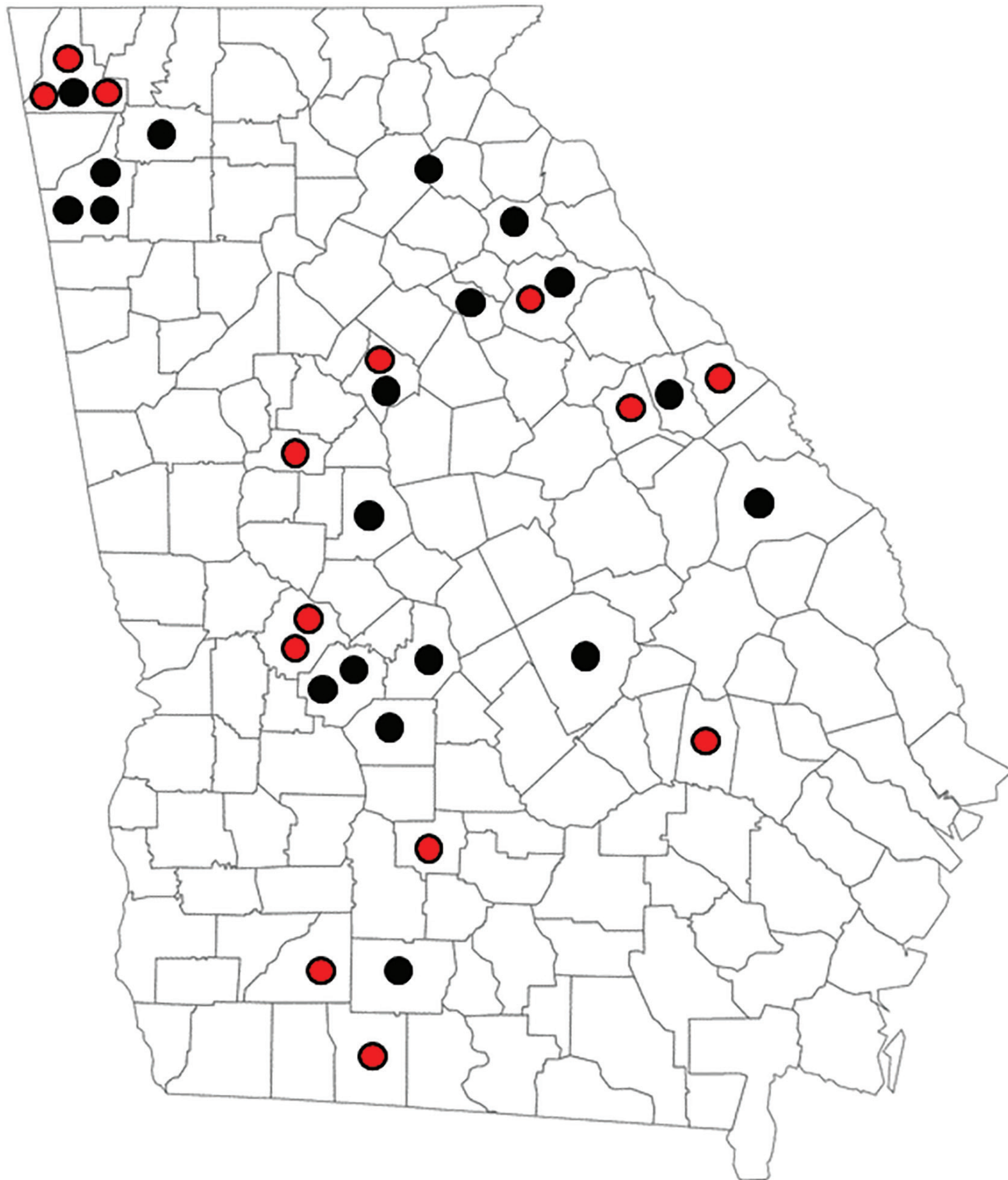

Table 1: Cross-classification of cELISA and PCR *Anaplasma* testing results for a subset of 479 Georgia beef cattle that were tested by both methods.

	cELISA result		
PCR result	**Negative**	**Positive**	**Total**
Negative	406	17	423
Positive	0	56	56
Total	406	73	479

Evaluation of potential risk factors

Producer responses to a herd management questionnaire are summarized in Table 2, along with univariable comparisons of the estimated *Anaplasma* seroprevalences in Georgia beef herds with different characteristics. In the univariable analysis, seroprevalence was positively associated with having a previous diagnosis of anaplasmosis on the operation; using CTC for the control of anaplasmosis; administering 4 or more injections of any kind per adult beef cow in the previous 12 months; using topical fly control products; bringing new cattle onto the operation in the previous 3 years; and experiencing cattle deaths or abortions during the previous 12 months.

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Table 2: Univariate associations between herd characteristics and *Anaplasma* seroprevalence based on testing of 1,059 adult cattle from 33 Georgia beef operations.

Variable	† No. Herds	Seroprevalence (95% CI)	‡*P*
Herd size			0.86
1-49 cows	10	7.5 (2.2, 22.3)	
50-199 cows	16	10.6 (3.1, 30.4)	
200 or more cows	7	6.4 (1.2, 28.3)	
Familiarity with anaplasmosis			0.46
Never heard of it / recognize name	15	5.9 (1.2, 24.6)	
Know some basics / fairly knowledgeable	18	10.9 (5.1, 21.7)	
Has anaplasmosis ever been diagnosed on this operation?			0.015
No	27	4.9 (1.5, 15.2)	
Yes	6	23.8 (13.6, 38.4)	
Was oral chlortetracycline used to control anaplasmosis in the last 12 months?			0.015
No	27	4.9 (1.5, 15.2)	
Yes	6	23.8 (13.6, 38.4)	
Were any of the following procedures used on this operation in the last 12 months?			
Mechanical dehorning			0.71
No	26	9.0 (3.8, 19.9)	
Yes	7	6.4 (1.2, 28.6)	
Castration with a knife			0.14
No	10	16.8 (5.2, 42.5)	
Yes	23	5.7 (2.2, 14.2)	
Ear notching			0.22
No	28	9.5 (4.3, 20.0)	
Yes	5	3.1 (0.6, 15.4)	
Tattoo			0.91
No	18	8.7 (2.7, 24.7)	
Yes	15	8.1 (3.3, 18.6)	
Growth implant			0.60
No	21	7.2 (3.2, 15.2)	
Yes	12	10.5 (2.7, 32.8)	
Were tools used to perform the procedures above always disinfected between animals?			0.31
No	16	5.9 (1.3, 23.8)	
Yes	13	13.6 (6.2, 27.4)	

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Table 2 Con't:

Variable	†No. Herds	Seroprevalence (95% CI)	‡*P*
On average over the last 12 months, how many injections of any kind were received by each:			
Beef cow			0.037
0-3	18	3.4 (1.0, 10.9)	
4 or more	13	16.6 (6.7, 35.5)	
Unweaned calf			0.16
0-3	15	5.0 (0.9, 22.4)	
4 or more	15	14.9 (8.6, 24.7)	
Is the same needle ever used to inject more than one animal?			0.86
No	4	9.7 (1.8, 38.4)	
Yes	29	8.3 (3.5, 18.1)	
During the last 12 months, were the following fly control methods used on this operation?			
Environmental (sprays, foggers, strips, zappers)			0.86
No	22	8.0 (3.6, 16.7)	
Yes	11	9.3 (1.8, 37.0)	
Topical (dust bags, dips, sprays, backrubs)			< 0.001
No	13	0.7 (0.2, 2.7)	
Yes	20	14.5 (6.9, 28.1)	
Insecticide treated ear tags			0.15
No	17	4.3 (1.3, 13.1)	
Yes	16	12.7 (4.9, 28.8)	
Oral products (e.g., feed-throughs)			0.22
No	22	5.9 (2.3, 14.2)	
Yes	11	14.5 (4.4, 38.2)	
Does this operation have one defined breeding season, two or more seasons, or no set breeding season?			0.042
One season	21	11.6 (5.1, 24.3)	
Two or more seasons	7	0.8 (0.1, 5.1)	
No set season	5	7.4 (1.3, 33.3)	
Which of the following best describes the breeding herd for this operation?			0.48
Seed stock	5	13.7 (5.1, 32.1)	
Commercial	18	5.6 (1.8, 16.6)	
Both	10	11.2 (2.6, 37.2)	
Did you bring any new cattle onto this operation in the last 3 years?			0.013
No	6	0.6 (0.1, 4.8)	
Yes	27	10.3 (4.8, 20.6)	

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Table 2 Con't:

Variable	†No. Herds	Seroprevalence (95% CI)	‡P
In the last 12 months, did you use the services of a veterinarian for cattle on your operation?			0.56
No	2	12.1 (3.9, 32.2)	
Yes	31	8.2 (3.7, 17.6)	
In the last 12 months, has your herd experienced:			
Abortions			0.037
No	17	3.5 (1.0, 11.4)	
Yes	16	13.8 (6.2, 28.0)	
Bovine respiratory disease			0.86
No	26	8.0 (3.9, 15.8)	
Yes	7	9.5 (1.4, 44.5)	
Cattle deaths			0.004
No	15	1.9 (0.7, 5.3)	
Yes	18	13.3 (6.0, 26.8)	

† Herd sample sizes vary due to item nonresponse.

‡ Wald *P*-values from univariable survey logistic regression models adjusted for sampling weights, herd-level clustering and stratification by herd size.

Seroprevalence was negatively associated with having 2 or more defined breeding seasons per year. The age and sex of individual cattle were not consistently recorded, so associations with these cow-level characteristics were not considered in the statistical analysis. Age was recorded for 477 cattle, with *Anaplasma* antibodies being detected in 6 (4.4%) of 138 cattle aged 2 to 3 years, 30 (12.7%) of 236 cattle aged 4 to 6 years, and 18 (17.5%) of 103 cattle aged 7 or more years. Sex was recorded for 852 cattle, with no *Anaplasma* antibodies being detected in the 31 animals that were identified as bulls.

Results of a multivariable logistic regression analysis to evaluate the associations between herd characteristics and *Anaplasma* seroprevalence are summarized in Table 3. In the multivariable analysis, seroprevalence was positively associated with the number of injections given to beef cattle in the previous 12 months and with bringing new cattle onto the operation in the previous 3 years. The seroprevalence was negatively associated with having 2 or more defined breeding seasons per year relative to having 1 or no set breeding season per year. A summary goodness of fit test indicated that the final multivariable model provided a good fit to the data ($P = 0.62$).

Discussion

In the current study, the true prevalence of *Anaplasma* infection in Georgia beef cattle was estimated to be 6.9% (95% CI: 2.0%, 16.3%). This is a somewhat higher estimate than that reported in another recent survey, which estimated that the true prevalence of *Anaplasma* infection in Georgia beef cattle was 2.6%.[19] That study was based on a sample of 293 cull beef cows from one cattle auction and one slaughterhouse, with 82% of the samples originating from a single county.

The southeastern United States is considered to be endemic for anaplasmosis, and the disease has been known to exist in Georgia for more than 80 years.[21] However, variances in estimated true seroprevalence rates have been reported in recent surveys from the southern states. An investigation of the prevalence of *A. marginale* among 1,085 cattle from 12 different herds in Florida determined that the prevalence of the disease varied from 2.6% to 85%. As the overall seropositive rate of 50.3% was higher than the apparent statewide estimated *A. marginale* seroprevalence of 20.32%, they suggested that the endemic stability of BA in Florida should not be presumed.[22] In another study from the southeastern U.S.,[23] the regional seroprevalence for *A. marginale* in beef cows sampled from slaughter plants was 13.0% and ranged from 2.44% to 35.18%. In a Mississippi study, the estimated true seroprevalence of BA was 29.02% (95% CI: 22.74 to 36.07%) with cELISA from an active survey of 207 beef cows slaughtered between May 2013 and December 2014.[24] Additionally, they reported the estimated true seroprevalence of 21.62% (95% CI: 20.18 to 23.11%) from reviewing 5,182 specimen records submitted for BA cELISA testing between 2002 and 2018. The cELISA based seroprevalence of bovine *Anaplasma* in the upper midwestern U.S. over a 10-year period (2001 to 2010) was 6.8% out of 226,923 samples tested at the Veterinary Diagnostic Laboratory, University of Minnesota.[25] A recent study reported anaplasmosis to be present in 52.5% Kansas beef cow-calf herds (prevalence ranged from 19.1% of herds in western Kansas to 87.3% of herds in eastern Kansas) and selected management practices were found to be associated with herd infection status.[26] Also, analysis of cELISA data for the assessment of within-herd seroprevalence of *A. marginale* antibodies in an Iowa dairy herd revealed that 38% of the animals tested positive for BA.[6] These variations in seroprevalence of anaplasmosis between the states could serve as a contributing factor in disease transmission.

The cELISA identified a significantly higher percentage of positive results than the PCR test ($P < 0.001$). A similar pattern of detecting more cELISA positive samples than PCR positive samples was recently reported by Parvizi et al. where 18. 5%

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Table 3: Multivariable survey logistic regression model for herd characteristics associated with *Anaplasma* seroprevalence. Complete information was available for 1,019 adult cattle from 31 Georgia beef operations.

Variable	Coefficient (SE)	Odds ratio (95% CI)	*P
On average over the last 12 months, how many injections of any kind were given to each beef cow?			
0-3	Reference	Reference	
4 or more	2.23 (0.75)	9.3 (2.0, 43)	0.006
Does this operation have one defined breeding season, two or more seasons, or no set breeding season?			
One season or No set season	Reference	Reference	
Two or more seasons	-3.48 (1.05)	0.03 (0.004, 0.26)	0.002
Did you bring any new cattle onto this operation in the last 3 years?			
No	Reference	Reference	
Yes	3.53 (1.05)	34 (4.0, 294)	0.002
Constant term	-6.52 (1.18)	NC	< 0.001

* Wald P-values from a multivariable survey logistic regression model adjusted for sampling weights, herd-level clustering and stratification by herd size.

NC - Not calculated because the odds ratio for the constant term is not meaningful.

of the bovine samples were cELISA positive and only 5.3% samples were positive by PCR.[27] Occurrence of more seropositive than PCR positive samples in our study may be due to a couple of factors: 1) samples were collected over a 14-month period (between April 2018 and June 2019) which included the vector season and the non-vector season; and 2) PCR-positive samples are usually observed in more acute cases whereas seropositivity could be due to recent infection or past exposure.

In the current study, samples were collected from 33 herds in 24 different counties. While the herds participating in the current study were more widely distributed across the state than those in the study by Okafor et al.,[19] they were selected by convenience from among producers who were willing to participate. Consequently, it is difficult to know whether these herds are truly representative of the entire population of Georgia beef cattle. The population-level prevalence of *Anaplasma* infection in Georgia beef cattle may be higher or lower than that estimated in the current study. Nonetheless, the current study does provide evidence that *Anaplasma* infection is widely distributed in beef herds across the state and suggests that additional efforts are warranted to educate producers about this important pathogen.

In the multivariable analysis, *Anaplasma* seroprevalence was positively associated with the number of injections given to beef cattle in the previous 12 months and with bringing new cattle onto the operation in the previous 3 years. Giving cattle a larger number of injections may represent a greater risk of transmitting bloodborne pathogens, especially considering that using the same needle to inject multiple animals was reported to be a common practice in the participating herds. Likewise, bringing new cattle onto an operation may represent an increased risk of importing 1 or more infected animals. *Anaplasma* seroprevalence was negatively associated with having 2 or more defined breeding seasons per year, relative to having 1 or no set breeding season. The reasons for this finding are unclear. This may represent a spurious result, since only 7 herds indicated that they had 2 or more defined breeding seasons, and *Anaplasma* was only detected on 1 of these 7 operations. Alternatively, having 2 breeding seasons may be consistent with having a higher overall level of management. Regardless, all of the associations identified in the current study should be interpreted with caution. The analysis was exploratory, the number of participating herds was small, and the cross-sectional nature of the study precludes one from distinguishing between characteristics that might increase the risk for *Anaplasma* infection and those that might be a consequence of *Anaplasma* infection.

Conclusion

The results of the estimated true prevalence of *Anaplasma* infection in Georgia beef cattle from the current study reflect a wide distribution of anaplasmosis across the state of Georgia. This study also revealed a correlation between some management practices with BA infection status. Further studies are warranted to investigate the economic impact and the variants of *A. marginale* across the state of Georgia. As the burden of BA apparently extended throughout the state, it is recommended that the prevention and control measures could be intensified across the state by focusing on the identified putative risk factors.

Endnotes

[a] Anaplasma Antibody Test Kit, cELISA, VMRD Inc, Pullman, WA

[b] MagMax Pathogen RNA/DNA Kit, ThermoFisher Scientific, Waltham, MA

[c] Qiagen BioSprint 96 Magnetic Particle Processor, ThermoFisher Scientific, Waltham, MA

[d] Xeno Internal Positive Control DNA, ThermoFisher Scientific, Waltham, MA

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[e] PCR Primers and Probe, IDT Inc., Coralville, Iowa

[f] TaqMan Universal PCR Master Mix, Applied Biosystems, ThermoFisher Scientific, Waltham, MA

[g] VetMAX Xeno IPC-VIC Assay, ThermoFisher Scientific, Waltham, MA

[h] ABI 7500 Real-time PCR instrument, ThermoFisher Scientific, Waltham, MA

[i] Stata version 16.1, StataCorp LLC, College Station, TX

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Conflict of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

1. Kocan KM, de la Fuente J, Blouin EF, et al. The natural history of *Anaplasma marginale*. *Vet Parasitol* 2010;167:95-107.

2. Kocan KM, de la Fuente J. Co-feeding studies of ticks infected with *Anaplasma marginale*. *Vet Parasitol* 2003;112:295-305.

3. Coarsey MD, Wilkes RP, Jones L, et al. A retrospective study of bovine anaplasmosis cases in Georgia: 2009-2017. Abstract in: *Proc of the Am Assoc of Vet Lab Diag* 2018;36.

4. Reppert EJ. Review of current anaplasmosis control strategies and future directions. *Proc Am Assoc Bov Pract Conf* 2019;71-72.

5. Coetzee JF. Bovine anaplasmosis. *Proc Am Assoc Bov Pract Conf* 2017;28-31.

6. Curtis AK, Coetzee JF. Assessment of within-herd seroprevalence of *Anaplasma marginale* antibodies and associated decreased milk production in an Iowa dairy herd. *Appl Ani Sci* 2021;37:126-131.

7. Aubry P, Geale DW. A Review of Bovine Anaplasmosis. *Transbound Emerg Dis* 2011;58:1-30.

8. Kocan KM, De La Fuente J, Blouin EF, et al. *Anaplasma marginale* (Rickettsiales: Anaplasmataceae): recent advances in defining host–pathogen adaptations of a tick-borne rickettsia. *Parasitology* 2004;129:S285-S300.

9. Scoles GA, Broce AB, Lysyk TJ, et al. Relative efficiency of biological transmission of *Anaplasma marginale* (Rickettsiales: Anaplasmataceae) by *Dermacentor andersoni* (Acari: Ixodidae) compared with mechanical transmission by *Stomoxys calcitrans* (Diptera: Muscidae). *J Med Entomol* 2005;42:668-675.

10. Richey E. Bovine anaplasmosis. *Proc Am Assoc Bov Pract Conf* 1991;3-11.

11. Richey E, Palmer G. Bovine anaplasmosis. *Compend Contin Educ Pract Vet* 1990;12:1661-1668.

12. Potgieter F, Van Rensburg L. The persistence of colostral *Anaplasma* antibodies and incidence of in utero transmission of *Anaplasma* infections in calves under laboratory conditions. *Onderstepoort J Vet Res* 1987;54:557-560.

13. Coetzee JF, Apley MD, Kocan KM, et al. Comparison of three oxytetracycline regimens for the treatment of persistent *Anaplasma marginale* infections in beef cattle. *Vet Parasitol* 2005;127:61-73.

14. Reinbold JB, Coetzee JF, Hollis LC, et al. The efficacy of three chlortetracycline regimens in the treatment of persistent *Anaplasma marginale* infection. *Vet Micro* 2010;145:69-75.

15. Carelli G, Decaro N, Lorusso A, et al. Detection and quantification of *Anaplasma marginale* DNA in blood samples of cattle by real-time PCR. *Vet Micro* 2007;124:107-114.

16. Landis JR, Koch GG. An application of hierarchical kappa-type statistics in the assessment of majority agreement among multiple observers. *Biometrics* 1977:363-374.

17. Dean N, Pagano M. Evaluating confidence interval methods for binomial proportions in clustered surveys. *J Surv Stat Methodol* 2015;3:484-503.

18. Rogan WJ, Gladen B. Estimating prevalence from the results of a screening test. *Am J Epidemiol* 1978;107:71-76.

19. Okafor CC, Collins SL, Daniel JA, et al. Seroprevalence of bovine anaplasmosis in Georgia. *Vet Parasitol: Reg Stud Reports* 2019;15:100258.

20. Archer KJ, Lemeshow S. Goodness-of-fit test for a logistic regression model fitted using survey sample data. *Stata J* 2006;6:97-105.

21. Stiles GW. Anaplasmosis: a disease of cattle. *Yearbook of Agriculture: USDA*, 1942;579-587.

22. Curtis AK, Whitlock BK, Daniel JA, et al. Assessment of statewide and within-herd seroprevalence of *Anaplasma* marginale antibodies in 12 Bos taurus–Bos indicus cow herds and the association with sporadic outbreaks of bovine anaplasmosis in Florida. *Appl Ani Sci* 2021;37:689-696.

23. Whitlock B, Daniel J, Harvey B, et al. Seroprevalence of bovine anaplasmosis in the southern US. *Proc Am Assoc Bov Pract Conf* 2014 Sep 18.

24. Okafor CC, Collins SL, Daniel JA, et al. Factors associated with seroprevalence of bovine anaplasmosis in Mississippi, USA. *Vet Parasitol: Reg Stud Rep* 2019;17:100301.

25. Durrani A, Goyal S. A retrospective study of *Anaplasma* in Minnesota cattle. *Turk J Vet Anim Sci* 2012;36:131-136.

26. Spare MR, Hanzlicek GA, Wootten KL, et al. Bovine anaplasmosis herd prevalence and management practices as risk-factors associated with herd disease status. *Vet Parasitol* 2020;277:100021.

27. Parvizi O, El-Adawy H, Melzer F, et al. Seroprevalence and molecular detection of bovine anaplasmosis in Egypt. *Pathogens* 2020;9(1):64.

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