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Original Article

Factors associated with seroprevalence of bovine anaplasmosis in Mississippi, USA

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ABSTRACT

Anecdotally, Veterinary Feed Directive prescriptions in the southeastern United States (U.S.) are written most often for treatment and prevention of bovine anaplasmosis (BA) but seroprevalence estimates and factors associated with this disease are currently unavailable in Mississippi (MS). Bovine anaplasmosis, a tick-borne disease of cattle caused by *Anaplasma marginale,* remains an economically important disease in U.S. The lack of recent seroprevalence of BA throughout the U.S. makes accurate assessment of production losses incurred by the cattle industry in the U.S. difficult, if not impossible to estimate. This study was aimed at determining the seroprevalence of and factors associated with BA in MS. Data were obtained from an active survey of 207 beef cows slaughtered between May 2013 and December, 2014 as well as from reviewing 5182 Veterinary Diagnostic Laboratories (VDLs) records of specimens from MS submitted for BA testing between 2002 and 2018. From the active surveillance, the overall observed apparent seroprevalence of BA in MS with cELISA was 28.99% (95% CI: 23.23 — 35.50%) while the estimated true seroprevalence was 29.02% (22.74 — 36.07%). However, from the laboratory records, the overall apparent period seroprevalence of BA in MS between 2002 and 2018 irrespective of diagnostic assay used was 16.72% (15.73 — 17.76%) and yearly increase in the diagnosis of BA followed a significant trend ($P < 0.0001$). With cELISA, the apparent seroprevalence of BA was 22.11% (20.78 — 23.49%) and the estimated true seroprevalence was 21.62% (20.18 — 23.11%). However, with CFT, the apparent seroprevalence of BA was 13.50% (10.75 — 16.81%) and the estimated true seroprevalence was 47.90% (36.30 — 61.87%). Factors associated with positive BA results were age, cattle type, and quarter of the year the specimens were submitted. The odds of the outcome were 22 as high in adults, 27 times as high in beef cattle, and 2 times as high between October to December in comparisons to juveniles, dairy cattle, and between April to June, respectively. Cattle population in the counties was not associated with positive BA results. Current records from the VDLs appear to accurately estimate the seroprevalence of BA in MS and thus serves as a reliable surveillance tool BA in the state. Because the burden of BA appears to be distributed throughout the state, future prevention and control measures for BA should focus on the identified putative risk factors and be intensified throughout MS.

Abbreviations: AAVLD, American Association of Veterinary Laboratory Diagnosticians; BA, Bovine anaplasmosis; CFT, Complement fixation test; cELISA, Competitive Enzyme-linked immunosorbent assays; MS, State of Mississippi; MVRDL, Mississippi Veterinary Research and Diagnostic Laboratory in Pearl, MS; Se, Sensitivity; Sp, Specificity; TVMDL, Texas A&M Veterinary Medical Diagnostic Laboratory; 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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1. Introduction

Bovine anaplasmosis (BA), caused by *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 vertically (across the placenta) (Aubry and Geale, 2011; Kocan et al., 2010; Radostits and Done, 2007). In the U.S., interstadial transmission of *A. marginale* has been demonstrated by the 3-host ticks, *Dermacentor andersoni* and *Dermacentor variabilis* whereas about 20 species of ticks have been implicated as vectors worldwide (Kocan 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. The lack of recent information regarding the prevalence of BA throughout the U.S. makes accurate assessment of production losses incurred by the cattle industry in the U.S. difficult, if not impossible.

Management practices toward BA control include increased diagnostics, control of vector and cattle movement, reducing iatrogenic transmission, and administration of low doses of tetracycline antimicrobials in feed (Aubry and Geale, 2011). Cattle that recover from acute anaplasmosis, including those treated with recommended doses of tetracycline, maintain lifelong undulating bacteremia that can be difficult to detect in smear when bacteria numbers are low (Aubry and Geale, 2011; Eriks et al., 1989; Kocan et al., 2010; Palmer et al., 2000; Radostits and Done, 2007; Richey and Palmer, 1990). Although deaths may still occur, persistent infections usually confer resistance to clinical anaplasmosis (Kocan et al., 2010). Persistently infected cattle serve as reservoirs through which vectors introduce *A. marginale* into naive cattle populations, thereby leading to the endemicity of BA (De Echaide et al., 1998; Futse et al., 2003; Reeves and Swift, 1977).

Anecdotally, Veterinary Feed Directive prescriptions in southeastern U.S. are written most often for treatment and prevention of BA. Specifically, beef cattle producers in Tennessee consider BA as in important disease driving their use of antibiotics (Ekakoro et al., 2019). These antibiotics in cattle can be administered for long periods. However, 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 possible effects of BA on judicious use of antibiotics in cattle in the southern U.S. The last reported prevalence of BA in the greater southern U.S. region occurred in the 1970's, ranged from 2% to 24%, and Mississippi (MS) recorded the highest seroprevalence (McCallon, 1973). However, complement fixation test (CFT) used to determine the seroprevalence has a lower sensitivity than newer diagnostic tests for BA (Aubry and Geale, 2011; Coetzee et al., 2007). Estimating the current seroprevalence of BA in MS is therefore a critical first step to implementing appropriate BA control programs in this state and can be a good indicator for the highest seroprevalence estimate in the region.

The objective of this study was to estimate the temporal seroprevalence and risk factors associated with A. *marginale* infections in MS cattle through active purposive screening of beef cows as well as the use of a 16-year accredited Veterinary diagnostic laboratories (VDLs) records. The expected results would provide (1) insights into the reliability of laboratory records in estimating the seroprevalence of BA in MS, and (2) farmers and policy makers the benchmark seroprevalence estimates needed to improve the control of BA in MS. 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 MS beef cows for BA was performed as previously described (Okafor et al., 2018b). Descriptively, based on a population of 495,381 beef cows (NASS, 2014), an estimated seroprevalence of 24% and a confidence level of 95%, 280 beef cows were required to estimate the seroprevalence of BA in MS beef cows. This sample size was calculated using the Epi Info™ Version 7.0 software (Centers for Disease Control and Prevention, Atlanta, GA, USA). Two slaughterhouses that slaughtered a significant portion of beef cattle from MS were purposively selected as specimen collection sites. These slaughterhouses, Southeastern Provision and FPL Food, were located in Bean Station, Tennessee and Augusta, Georgia, respectively. Between May to August 2013 (Southeastern Provision) and August to December 2014 (FPL Food), blood specimens were collected from cull beef cows at these slaughterhouses. Specimens were collected only from cows with a USDA-approved backtag identification beginning with the prefix “65”, indicating MS 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 blood collection tube (BD Vacutainer Serum Separator; 8.5 mL). All blood specimens were transported in ice-pack containers and tested with competitive enzyme linked immunosorbent assay (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 sample submissions for BA diagnosis performed between January 2002 and August 2018 were requested from two American Association of Veterinary Laboratory Diagnosticians (AAVLD) accredited VDLs in Texas (TX) and one in MS. 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), and the Mississippi Veterinary Research and Diagnostic Laboratory in Pearl, MS (MVRDL). Obtained records included date of specimen submission, geographic information (county associated with the submission), breed, type of cattle, sex, age, the diagnostic assay used, testing location, and the test result. Cattle breeds with < 70 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 VDLs were card test, CFT, and cELISA. To facilitate analysis, all submissions without definite positive or negative results (suspect, anticomplementary, or missing) were removed.

2.3. Analysis

First, an overall apparent seroprevalence of BA for the study period was presented irrespective of the diagnostic assay used. Subsequently, the yearly temporal distribution of these seroprevalence estimates was presented. Then, seroprevalence estimates were presented accounting for the diagnostic assay used. In estimating the true seroprevalence of BA, previously described sensitivity (Se) and specificity (Sp) results for CFT, cELISA (Aubry and Geale, 2011; Coetzee et al., 2007), and card

test (Fosgate et al., 2010) were used. Utilized test parameters are as follows: CFT (Se = 26%; Sp = 98%), cELISA (Se = 95%; Sp = 98%), and card test (Se = 34%; Sp = 99%). True seroprevalence estimates were calculated as described previously (Reiczigel et al., 2010; Rogan and Gladen, 1978). Wilson's confidence intervals were calculated on the assumption that Se and Sp were known exactly as described previously (Reiczigel et al., 2010). To access the reliability of the VDLs in estimating the seroprevalence of BA in MS, estimates from the VDLs were compared with that from the slaughter survey. For a fair comparison between the two types of surveillance, seroprevalence estimates with cELISA between May and August for 2013, and from August to December for 2014 were calculated. While using the same diagnostic assay, it was necessary to match the same season the slaughter data was obtained because season of the year is known to be associated with seroprevalence of BA (Okafor et al., 2018a; Okafor et al., 2018b). Cattle population data for each county in MS were obtained from the 2012 census of the National Agricultural Statistical Service (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. Choropleth maps were created to display data in a visually concise format based on the slaughter survey, state-wide diagnostic laboratory data, and 2012 census of the National Agricultural Statistical Service using ArcGIS 10.5 (ESRI, Redlands, CA).

Both univariable and multivariable logistic regression analyses were performed to test the effects of cattle age (adult or juvenile), sex, breed and breed category/cattle type (beef, dairy, or mixed), screening test type (cELISA, CFT), test laboratory, year and month of specimen submission on positive diagnosis of BA. Cochran-Armitage test for trend was used to assess any yearly trend in the diagnosis of BA. These data analyses were conducted in SAS9.4 for windows 64× (Cary, NC). Odds ratios and their CIs were used to measure the strength of associations between the explanatory variables and the outcome. A P value of ≤ 0.05 was considered significant. In fitting the final multivariable logistic model, all the variables in the univariable analyses were examined and interactions between selected variables were tested. For variables that measured similar characteristics (e.g. breed and breed category), only 1 of the variables was used in the final model building based on ease of biological interpretation. The tested interactions were: sex and breed; age and sex; age and breed; and year and quarter of the year. A confounding variable was defined as a non-intervening variable that changed the coefficient of a previously significant variable in the logarithm scale by at least 20% (Dohoo et al., 2009). The overall assessment of the final model was done using the Bayesian Information Criteria (BIC).

3. Results

In the active BA beef cow screening, 207 beef cows originating from 16 (19.51%) of the 82 counties in MS were sampled (Fig. 1, Table 1). Of the 207 beef cows, 60 were positive and 147 were negative for BA. Hence, the overall observed apparent seroprevalence of BA in MS was 28.99% (95% CI: 23.23 — 35.50%) while the estimated true seroprevalence was 29.02% (95% CI: 22.74 — 36.07%). But, the county level apparent seroprevalence ranged from 0 to 100%. 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 29 stockyards in 22 counties approved to sell cattle in MS during the survey, and beef cows originated from 18 (62.07%) and 16 (72.73%) of those stockyards and counties, respectively. The number of beef farms were significantly higher (p = 0.001) among counties whose cattle were sampled when compared to those that were not (Table 2). However, among those counties whose cattle were tested for BA, there was no significant difference between those with and without positive results with respect to the total cattle population (p = 0.3785), number of beef farms (p = 0.4524), and number of dairy farms (p = 0.3258) (Table 2).

Between 2002 and October 2006, all BA testing record for MS were from the TVMDLs whereas from November 2006 to August 2018, most records came from the MVRDL and a few from the TVMDLs. Our collective database from the 3 VDLs had 5182 submissions for BA testing from MS. However, 32 submissions were deleted because of either missing or inconclusive results. Of the balance of 5150 submissions used in the analysis, 1564 submissions came from the TVMDLs database (830 from TVMDL-College Station and 735 from TVMDL-Amarillo) and 3585 submissions were from the MVRDL. There was no county of origin information on all 1564 results from the TVMDLs and the MVRDL specimens came from 59 (71.95%) of the 82 counties in MS (Table 3 and Fig. 2). The number of beef farms were significantly higher (p = 0.0003) among counties whose cattle were tested for BA at the MVRDL when compared to those that were not (Table 3), but no significance difference was observed in the total cattle population (p = 0.5546) nor the number of dairy farms (p = 0.9339). Among those counties whose cattle were tested for BA, there was no significant difference between those with and those without positive results with respect to the total cattle population (p = 0.5517), number of beef farms (p = 0.1252), and number of dairy farms (p = 0.4049).

Of these 5150 submissions, 861 were positive and 4289 were negative. Irrespective of diagnostic assay used, the overall apparent period seroprevalence of BA in MS between 2002 and 2018 was 16.72% (95% CI: 15.73 — 17.76%) and yearly increase in the diagnosis of BA followed a significant trend (P < 0.0001) but there was no increased trend in the total sample submissions over the period (Fig. 3). Regarding the assay used, 3596 (69.83%) specimens were tested with cELISA (795 positives and 2801 negatives); 1065 (20.68%) were tested with card test (all yielded negative results), and 489 (9.5%) were tested with CFT (66 positives and 423 negatives). With cELISA, the apparent seroprevalence of BA was 22.11% (95% CI: 20.78 — 23.49%) and the estimated true seroprevalence was 21.62% (95% CI: 20.18 — 23.11%). However, with CFT, the overall apparent seroprevalence of BA was 13.50% (95% CI: 10.75 — 16.81%) and the estimated true seroprevalence was 47.90% (95% CI: 36.30 — 61.87%).

Specifically, the apparent and estimated true seroprevalence of BA with cELISA between May to August 2013 was 12.07% (95% CI: 5.97 — 22.88%) and 10.83% (95% CI: 4.08 — 22.69%), respectively. Whereas, the apparent and estimated true seroprevalence of BA with cELISA between August to December, 2014 was 31.71% (95% CI: 22.65 — 42.40%) and 31.94% (95% CI: 22.0 — 43.71%), respectively. Collectively for the duration between May to August 2013 and August to December 2014, the apparent and estimated true seroprevalence of BA with cELISA was 23.57% (95% CI: 17.31 — 31.25%) and 23.2% (95% CI: 16.19 — 31.60%), respectively.

At the univariable logistic regression analysis (Table 4), the following variables were independently associated with the diagnosis of BA in MS cattle: cattle age, breed type (beef vs dairy), breed, type of assay used for testing (cELISA vs CFT), and laboratory that performed the test, year as well as the season (quarter) of year of testing. Card test yielded only negative results and was dropped in the analysis because it caused a quasi-complete separation of data points. Beef cattle, Angus, Holstein, and other breeds, cELISA, and tests performed at TVMDL-College Station and MVRDL were more likely to have a positive diagnosis of BA than dairy cattle, mixed cattle breeds, CFT, and tests performed at TVMDL-Amarillo, respectively. Furthermore, year as well as the season (quarter) of year of testing was associated with diagnosis of BA in MS. When compared to 2002, apparent seroprevalence of BA was significantly higher for each year from 2006 to 2018 and diagnosis of BA was more likely in the fourth, third, and first quarters of the year when compared to the second.

In the final multivariable logistic regression model fitted with 359 observations, the significant factors associated with BA diagnosis in MS

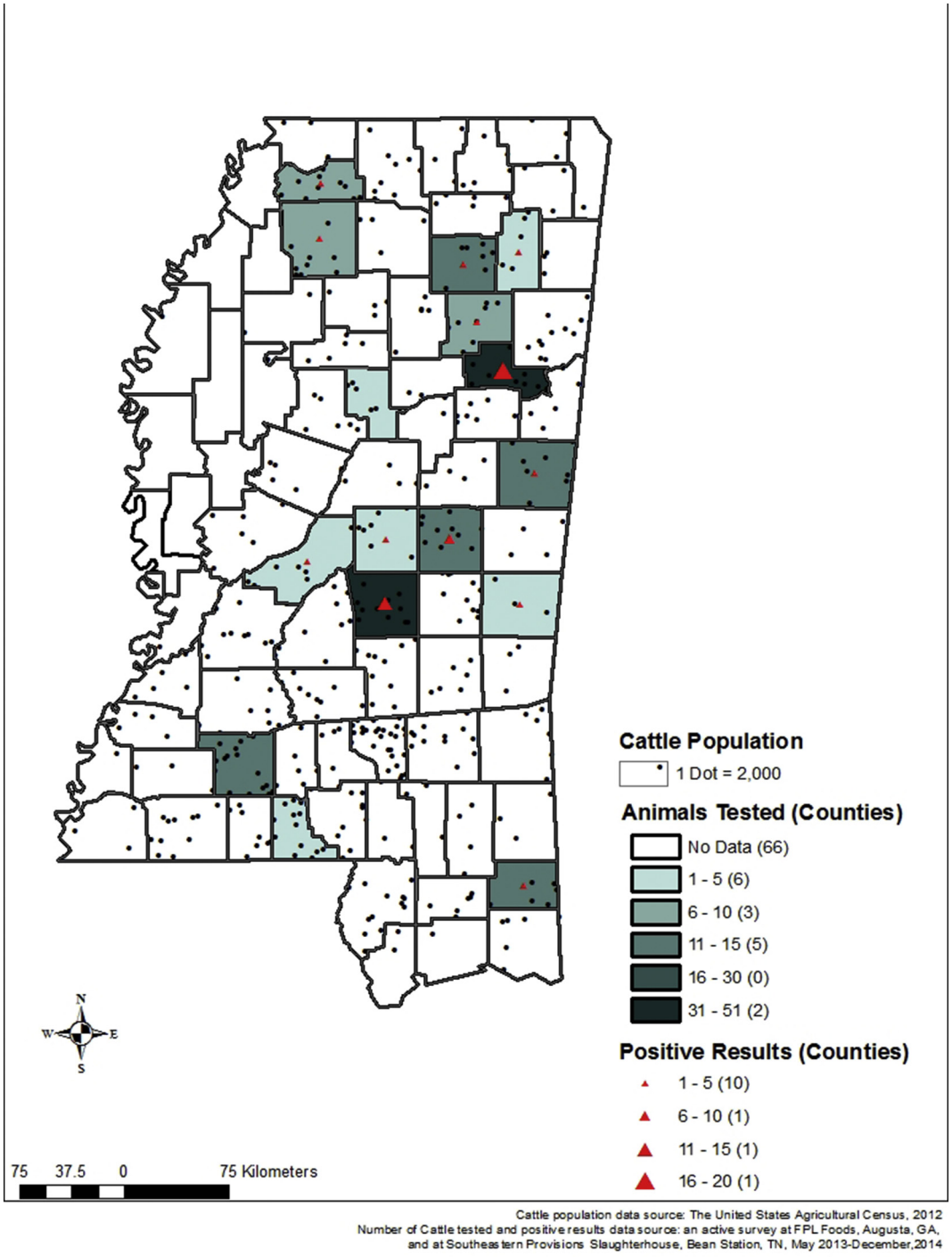

Fig. 1. Choropleth map of cattle population density per county in Mississippi, number of beef cows tested, and positive results and their distribution based on prospective surveillance data for bovine anaplasmosis between May to August 2013 (Southeastern Provision) and August to December 2014 (FPL Food).

were cattle age, breed type and season (quarter) of year of testing (Table 5). The odds of the outcome were 22 as high in adults, 27 times as higher in beef cattle, and 2 times as high between October and December in comparisons to juveniles, dairy cattle and between April and June, respectively. Although yearly increase in the diagnosis of BA followed a significant trend ($P < 0.0001$) (Fig. 3) and year was significantly associated with the diagnosis of BA in MS in the univariable analysis logistic regression analysis (Table 4), it was not significant in the final multivariable model.

4. Discussion

This study presents for the first time factors associated with the seroprevalence of BA in MS as well as the comparison between slaughter survey (active surveillance) using cELISA and the 16-year laboratory record evaluation (passive surveillance) for estimation of seroprevalence of BA in MS. The likelihood of a positive diagnosis for BA increased among adults, beef breeds, and samples obtained between October to December in comparisons to juveniles, dairy breeds, and April to June, respectively. Though all cattle ages are susceptible to infection by *A. marginale*, seroprevalence of BA have been demonstrated

Table 1
Apparent and estimated true seroprevalence of bovine anaplasmosis in Mississippi counties estimated with slaughter survey, between August 2013 and August to December 2014

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)
Madison	13,596	228	N/A	1 (1)	100	–
Neshoba	18,732	302	3	14 (8)	57.14 (32.59 — 78.62)	59.29 (31.95 — 83.22)
Chickasaw	16,299	209	N/A	7 (4)	57.14 (25.05 — 84.18)	59.29 (22.08 — 91.53)
Tate	19,907	232	8	10 (5)	50 (23.66 — 76.34)	51.61 (21.77 — 81.46)
Panola	17,281	220	N/A	8 (4)	50 (21.52 — 78.48)	51.61 (18.59 — 84.63)
Lee	9148	248	3	2 (1)	50 (9.45 — 90.55)	51.61 (0.57 — 98.56)
Clay	19,392	212	2	51 (17)	33.33 (21.97 — 47.03)	33.69 (20.80 — 48.64)
Pontotoc	13,043	274	5	15 (4)	26.67 (10.90 — 51.95)	26.52 (8.24 — 55.30)
Scott	21,222	351	4	46 (11)	23.91 (13.91 — 37.94)	23.56 (12.39 — 38.67)
Leake	14,888	292	1	5 (1)	20 (3.62 — 62.45)	19.35 (0 — 68.54)
Lauderdale	5215	135	3	5 (1)	20 (3.62 — 62.45)	19.35 (0 — 68.54)
Noxubee	16,138	195	3	14 (2)	14.29 (4.01 — 39.94)	13.21 (0.65 — 43.62)
George	12,663	291	N/A	12 (1)	8.33 (1.49 — 35.39)	6.81 (0 — 37.66)
Lincoln	33,667	314	11	12 (0)	< 0 (0 — 24.25)	< 0 (0 — 23.94)
Walthall	26,104	384	21	4 (0)	< 0 (0 — 48.99)	< 0 (0 — 54.53)
Montgomery	9037	142	1	1 (0)	< 0	–
Total	266,332	4029	65	207 (60)	28.99 (23.23 — 35.50)	29.02 (22.74 — 36.07)

N/A signifies not available. – signifies unable to estimated due to limited sample size.

to be significantly higher in adult cattle in comparison to juveniles (Aubry and Geale, 2011; Coetzee et al., 2005; Okafor et al., 2018a; Okafor et al., 2018b). Intuitively, the likelihood of being infected by vectors or through iatrogenicity increases as cattle gets older. With respect to breed type, beef cattle breeds are likely to have BA than dairy ones (Kocan et al., 2003; Okafor et al., 2018a; Okafor et al., 2018b). Compared to dairy cattle, beef cattle spend greater time in pasture than in barns and may be at increased risk of BA due to a higher opportunities of exposure to transmission vectors (Haskell et al., 2006; Simon et al., 2016). Quarter of year of specimen submission had an effect on seroprevalence of BA with a significant odds of disease observed in fall/early winter (October to December) but surprisingly not in the other seasons of the year. This observed effect of season on BA diagnosis in the present study differs from that observed in other southern U.S. states including, Oklahoma and Louisiana (Hugh-Jones et al., 1988; Rodgers et al., 1994), Kentucky (Okafor et al., 2018b), and TX (Alderink and Dietrich, 1983; Okafor et al., 2018a). Routinely, clinical outbreaks of BA occur predominantly during warm, wet seasons when vector-borne transmission is more common, due to optimal opportunities for exposure between naive animals and infected ticks (Alderink and Dietrich, 1983). During the typical summer months (June to August), Mississippi is usually hot with an average temperature of 100 °F compared to 97 °F in KY and 120 °F in TX (NIDIS, 2018a). But during the fall months (September to November), the average temperature in MS is 93 °F but 94 °F and 24 °F in KY and TX, respectively (NIDIS, 2018b). The temperature variations among these states may explain the observed differences in the effect of season on seroprevalence of BA. Particularly for MS, the summer climate may not be optimal for ticks' reproducibility and mobility whereas the fall climate may be more favorable for the ticks' activities, assuming tick vectors play a significant role in the epidemiology of BA in MS. However, in the absence of available information on tick season in MS, it is difficult to explain the factor responsible for the observed season effect. Alternatively, because iatrogenic *A. marginale* infection associated with contaminated surgical equipment or hypodermic needles may predispose clinical cases occurring outside the normal vector season (Reeves and Swift, 1977; Smith et al., 1989), iatrogenic transmission may be common in MS through cattle husbandry practices that allow shared hypodermic needles. Additional information would be useful in clarifying the speculative justifications for the seasonal difference in the seroprevalence of BA in MS. Finally, a few factors that were significant at the univariable logistic regression model (test type and test laboratory) could not be fitted in the multivariable model due to the limitations in the variable structure. As an example, the MVRDL used only cELISA and only captured observation from 2006 to 2018 whereas all BA testing record between 2002 and October 2006 were from the TVMDLs. Additional

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

Counties associated with cattle tested for bovine anaplasmosis (n = 16)

Variable	Minimum	Maximum	Mean	Lower 95% CL for mean	Upper 95% CL for mean	Standard deviation	Median
Cattle population	1266	9148	2980	1605	4356	2581	1906
Number of farms	151	414	274	234	315	77	249
Beef farms	135	384	252	215	289	107	240
Dairy farms	0	21	5	2	9	6	3

Counties not associated with cattle tested for bovine anaplasmosis (n = 66)

Variable	Minimum	Maximum	Mean	Lower 95% CL for mean	Upper 95% CL for mean	Standard deviation	Median
Cattle population	160	9837	3989	3206	4773	3111	2471
Number of farms	1	528	175	147	203	112	169
Beef farms	1	472	161	136	186	102	155
Dairy farms	0	15	4	3	6	4	3

Table 3
Distribution of cattle and farm demographics between counties associated with cattle tested for bovine anaplasmosis in Mississippi and those that were not in the Mississippi Veterinary Research and Diagnostic Laboratory record review (2006–2018).

Counties associated with cattle tested for bovine anaplasmosis (n = 59)

Variable	Minimum	Maximum	Mean	Lower 95% CL for mean	Upper 95% CL for mean	Standard deviation	Median
Cattle population	295	9837	3663	2917	4409	2838	2271
Number of farms	2	528	222	194	250	107	222
Beef farms	2	472	203	178	229	97	202
Dairy farms	1	21	5	3	6	4	3

Counties not associated with cattle tested for bovine anaplasmosis (n = 23)

Variable	Minimum	Maximum	Mean	Lower 95% CL for mean	Upper 95% CL for mean	Standard deviation	Median
Cattle population	160	9587	4122	2511	5733	3539	2142
Number of farms	1	328	124	82	166	97	140
Beef farms	1	302	115	76	154	90	122
Dairy farms	1	8	4	-4	13	4	3

studies could provide further explanation in the epidemiology of BA in MS.

Bovine anaplasmosis in the mid-western U.S., specifically Kansas, steadily increased between years 2005 to 2013 (Hanzlicek et al., 2016). However, in the present study year was not significantly associated with the diagnosis of BA in MS cattle in the final multivariable model, although yearly increase in the diagnosis of BA followed a significant trend ($P < 0.0001$) (Fig. 3). Albeit not significant, the estimated true seroprevalence from the active surveillance [29.02% (95% CI: 22.74 — 36.07%)] was slightly higher than that from the passive surveillance [21.62% (95% CI: 20.18 — 23.11%)]. The sampling frame of the slaughter survey could have contributed to an observed apparent seroprevalence value that is biased away from the null because these data were collected from adult cull beef cows and samples were obtained between May to December, both reported to be significantly associated with a positive BA result at the multivariable analysis in the present study as well as from previous studies (Aubry and Geale, 2011; Coetzee et al., 2005; Okafor et al., 2018a; Okafor et al., 2018b). More so, beef cows are retained longer than beef bulls because of the economics associated with production of calves. Usually, beef cows are culled when they are aged (> 10 years of age), have bad teeth, are infertile, or have some morbidity (NAHMS, 2010). All these increases the likelihood of obtaining a positive BA diagnosis from beef cows. Because the laboratory records include juveniles, beef bulls, dairy cows, and samples obtained in months when seroprevalence is relatively less, the obtained seroprevalence estimates are therefore lower in this population in comparisons to the slaughter sample. So, the closeness in seroprevalence estimates from both populations is suggestive that these laboratory records could be a reliable surveillance tool for estimating and controlling the true burden of BA in MS cattle.

The apparent seroprevalence of 24% obtained more than forty years ago (McCallon, 1973) appear differently from the estimates in the present study, depending on which diagnostic test was used in the estimation. From the laboratory records, the apparent seroprevalence of BA was 22.11% (95% CI: 20.78 — 23.49%) with cELISA, 13.50% (95% CI: 10.75 — 16.81%) with CFT, and 0% with card test since all tested samples yielded negative results. These discrepancies in seroprevalence estimates is probably due to differences in the diagnostic test attributes (Aubry and Geale, 2011; Coetzee et al., 2007; Okafor et al., 2018a). Each of the diagnostic test used have inherent limitations. Card test has been discontinued for BA testing due to its low sensitivity property and CFT is no longer considered a reliable test for BA for similar reason (Aubry and Geale, 2011; Coetzee et al., 2007). Commonly, cELISA is considered a better assay for BA diagnosis, albeit their vulnerability to possible false negatives during the initial stages of infection or false positives due to cross-reactivity with other *Anaplasma spp.* (Aubry and Geale, 2011; Coetzee et al., 2007). Such cross-reactivity was reported in Switzerland when cELISA was used to classify cattle infected with *A. marginale* and *A. phagocytophilum* (Dreher et al., 2005). However, no reports of natural infections of *A. phagocytophilum* has been reported in U.S. cattle (Lascola et al., 2009; Tinkler et al., 2012). Therefore, cELISA remains a current valuable assay for estimating the seroprevalence of *A. marginale* infection in U.S. cattle. It is not uncommon that card test was the mostly used test in the previous study (McCallon, 1973) given the year the study was conducted. If a seroprevalence of 24% was obtained from a supposed low sensitivity test where as a CFT in this present study yielded an apparent seroprevalence of 13.5%, then seroprevalence of BA in MS must have reduced significantly over the years. In general, it is difficult to compare results obtained 40 years apart due to several causes, including changes in cattle management practices, frequency of screening, parameters of the diagnostic test used, and disease control measures. The reason for this apparent decrease in seroprevalence is unclear but could be attributed to improved cattle management practices as well as the state's success in the BA control efforts.

The role of geographical cattle population on seroprevalence of BA in southern U.S. is contentious. In the present study, the 59 counties with specimen submissions for BA testing (median total cattle population of 2271) did not have a significant greater cattle population than the 23 counties without specimen submissions (median total cattle population of 2142) and similar results were obtained in Georgia (Okafor et al., 2019), where 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). Conversely, seroprevalence of BA has been shown to be significantly higher in Kentucky counties with median total cattle population of 21,000 compared to counties with median total cattle population of 6300 (Okafor et al., 2018b) and in Texas counties with median total cattle population of 38,000 compared to counties with median total cattle population of 14,000 (Okafor et al., 2018a). Specifically, the proportion of herds reporting clinical cases of BA in Texas increased as herd size increased (Alderink and Dietrich, 1983), indicating that large cattle herds in Texas appeared to sustain BA infection more persistently than smaller herds. In Louisiana, however, the seroprevalence of cattle was independent of herd size (Hugh-Jones et al., 1988). Because herd sizes were not obtained in the present study as well as in other studies with contentious results on the effect of cattle population on seroprevalence of BA (Okafor et al., 2019; Okafor et al., 2018a; Okafor et al., 2018b), the effect of herd size on seroprevalence of BA could not be evaluated. But the discrepancies on role of geographical cattle population on seroprevalence of BA suggests that strategies for control of BA should be specific for each state and not be targeted absolutely to counties with

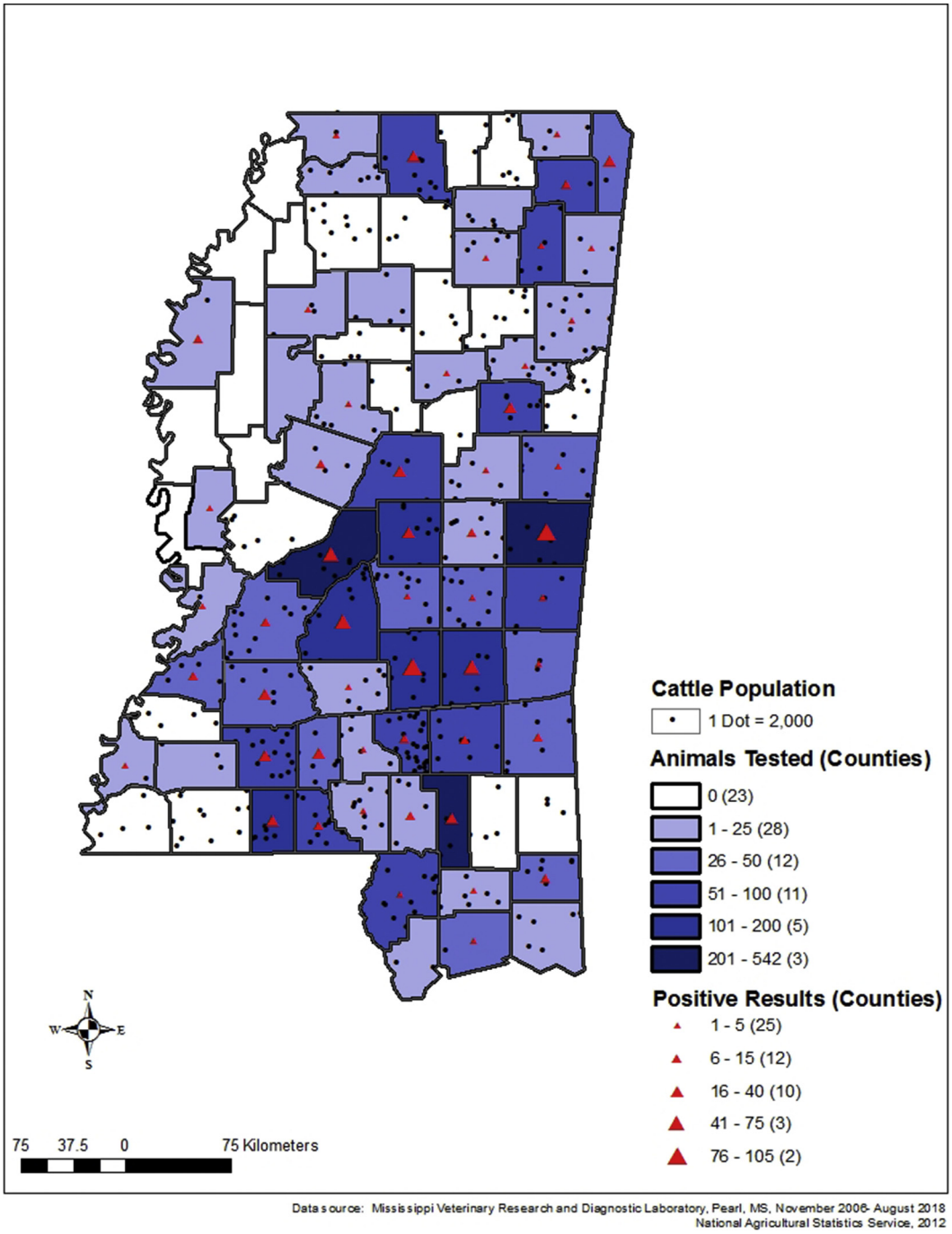

Fig. 2. Choropleth map of bovine anaplasmosis specimen submissions, positive results, and cattle population per county in Mississippi based on the Mississippi and Texas A&M Veterinary Diagnostic Laboratories data from 2002 to 2018 and National Agriculture Statistic Service data from 2012.

certain cattle population. Besides, these county seroprevalence estimates as observed in the present study may not necessarily reflect the true estimate in each county of residence of cattle because the TVMDLs records did not contain cattle's county of origin information but that of 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. The obtained estimates could be erroneous, if cattle are traded to another county after the backtag identification. Any such misclassification of cattle by county would likely be the same for counties irrespective of their total cattle population. Hence, such nondifferential misclassification bias would not have favored any specific county in MS and would be toward the null.

To emphasis the importance of BA burden in MS, the state had the highest apparent seroprevalence of BA of all the U.S. states evaluated in the last comprehensive study over 40 years ago (McCallon, 1973) and currently leads in seroprevalence over Texas, Kentucky, and Georgia. Notwithstanding that MS has fewer cattle population relative to Texas, Kentucky, and Georgia (NASS, 2014), it's relatively high seroprevalence is concerning. Given that minimum land surface temperature, diurnal temperature range, and humidity were specific climatic factors shown to increase the spread of BA in Kansas (Hanzlicek et al., 2016), the climatic conditions of MS may have supported its higher spatio-temporal distribution of BA. In actuality, the true seroprevalence in the

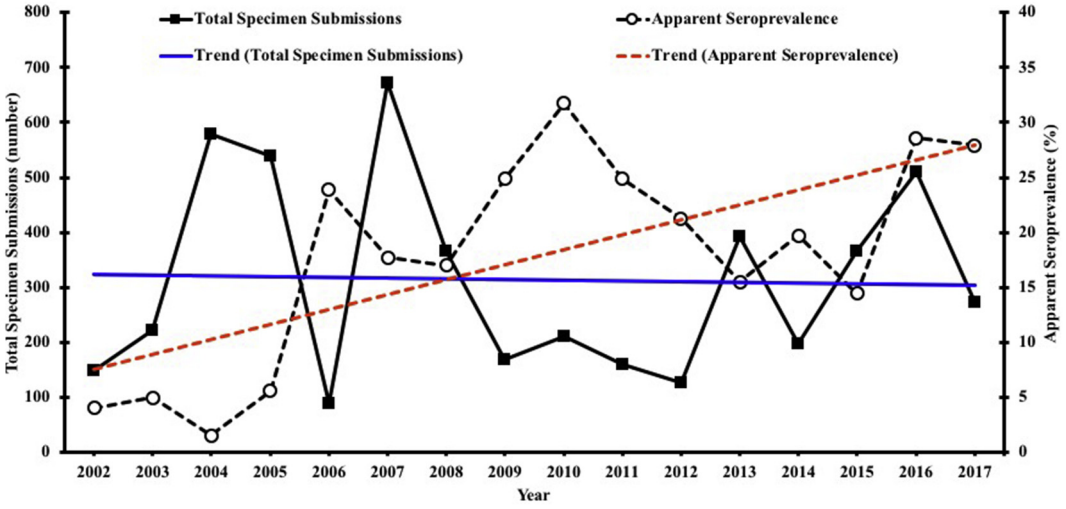

Fig. 3. Yearly time series of the average number bovine sample submissions and the apparent seroprevalence of bovine anaplasmosis in Mississippi from the Mississippi and Texas A&M Veterinary Diagnostic Laboratories data from 2002 to 2017.

population should be higher than the 29.02% obtained from the slaughter survey, because the actual number of samples used in the estimation fell short of the calculated sample size for the study. Nevertheless, future prevention and control measures for BA should be intensified throughout MS, because counties whose submissions had a positive BA diagnosis in both the slaughter survey (Fig. 1) and the laboratory records review (Fig. 2) appears distributed throughout the state.

5. Conclusion

Diagnosis of BA in MS was significantly higher among adults, beef breeds, and samples obtained between October to December in comparisons to juveniles, dairy breeds, and April to June, respectively.

Table 4
Logistic univariable analysis for associations between various factors and bovine anaplasmosis in Mississippi cattle at the Mississippi and Texas A&M Veterinary Diagnostic Laboratories, 2002–2018.

Variable	Category	No. of cattle	OR	95% CI	P value
Age	Juvenile	522	Referent	–	–
	Adult	2532	5.26	3.45 — 8.01	< 0.0001
Sex	Male	57	Referent	–	–
	Female	290	0.75	0.41 — 1.35	0.3350
Cattle type	Dairy	102	Referent	–	–
	Beef	371	5.66	2.41 — 13.34	< 0.0001
Breed	Mixed	261	Referent	–	–
	Angus	164	32.51	9.91 — 107	< 0.0001
	Holstein	78	7.17	1.75 — 29.35	0.0062
	Other	234	25.17	7.75 — 81.81	< 0.0001
Test type	CFT	489	Referent	–	–
	cELISA	3596	1.82	1.39 — 2.39	< 0.0001
Test laboratory	TVMDL- Amarillo	735	Referent	–	–
	TVMDL- College Station	830	9.81	4.22 — 22.81	< 0.0001
	MVRDL	3585	34.51	15.39 — 77.36	< 0.0001
Year	2002	148	Referent	–	–
	2003	222	1.23	0.45 — 3.41	0.6856
	2004	579	0.37	0.13 — 1.07	0.0659
	2005	538	1.40	0.57 — 3.42	0.4640
	2006	88	7.42	2.86 — 19.23	< 0.0001
	2007	672	5.09	2.20 — 11.80	0.0001
	2008	365	4.84	2.05 — 11.46	0.0003
	2009	169	7.83	3.22 — 19.03	< 0.0001
	2010	211	11.01	4.63 — 26.20	< 0.0001
	2011	161	7.82	3.21 — 19.08	< 0.0001
	2012	127	6.39	2.54 — 16.05	< 0.0001
	2013	393	4.35	1.84 — 10.29	0.0008
	2014	198	5.81	2.39 — 14.12	0.0001
	2015	366	4.01	1.68 — 9.53	0.0017
	2016	511	9.47	4.09 — 21.91	< 0.0001
	2017	272	9.18	3.89 — 21.66	< 0.0001
	2018	130	15.78	6.49 — 38.39	< 0.0001
Quarter of year	2	2078	Referent	–	–
	1	1459	1.76	1.35 — 2.29	< 0.0001
	3	910	2.78	2.22 — 3.47	< 0.0001
	4	703	4.15	3.42 — 5.03	< 0.0001

Table 5
Final multivariable logistic regression model of factors associated with diagnosis of bovine anaplasmosis in Mississippi cattle at the Mississippi and Texas A &M Veterinary Diagnostic Laboratories, 2002–2018.

Variable	Category	OR	95% CI	P value
Age	Adult vs Juvenile	22.14	2.99 — 164	0.0024
Breed type	Beef vs Dairy	26.79	3.63 — 198	0.0013
Quarter of year	4 vs 2	2.40	1.01 — 5.36	0.0327
	3 vs 2	1.15	0.51 — 2.62	0.7346
	1 vs 2	1.24	0.49 — 3.19	0.6507

Irrespective of diagnostic assay used, the overall apparent period seroprevalence of BA in MS from the laboratory records between 2002 and 2018 was 16.72% (95% CI: 15.73 — 17.76%) and yearly increase in the diagnosis of BA followed a significant trend but there was no increased trend in the total sample submissions over the period. With regards to the diagnostic assay used, the estimated true seroprevalence was 21.62% (95% CI: 20.18 — 23.11%) with cELISA and 47.90% (95% CI: 36.30 — 61.87%) with CFT. However, the estimated true seroprevalence with cELISA from the slaughter survey between 2013 and 2014 was 29.02% (95% CI: 22.74 — 36.07%). Current records from the VDLs appear to accurately estimate the seroprevalence of BA in MS and thus serves as a reliable surveillance tool BA in the state. Because the burden of BA appears to be distributed throughout the state, future prevention and control measures for BA should focus on the identified putative risk factors and be intensified throughout MS.

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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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.

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