Distribution trends of bovine *Anaplasma marginale* in Iowa, based upon veterinary diagnostic lab submissions from 2017-2022

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Abstract

Anaplasma marginale is the common etiological agent of bovine anaplasmosis. Infection results in erythrocyte destruction leading to the clinical signs of anemia, hypoxia, abortion, jaundice and sudden death. Cattle that survive this acute phase or have lower levels of initial bacteremia become chronic, persistent carriers. Carrier animals serve as reservoirs for the organism and allow infection to spread via mechanical or biological vectors, to susceptible herdmates. The objective of this study was to report the distribution trends of *A. marginale* positive test results during a 5-year period in Iowa. This study evaluated Iowa State University Veterinary Diagnostic Lab (ISU VDL) testing results from bovine cases submitted from January 1, 2017 to January 1, 2022, for molecular and serological detection of all stages of anaplasmosis. Cattle residing in Iowa with positive results for PCR or competitive ELISA were included in the analysis. The state was divided into 4 geographical districts (1 = northeast, 2 = southeast, 3 = southwest, and 4 = northwest) and the number of positive tests in each district was determined. Total positive tests by district included: northeast 282; southeast 277; southwest 223; northwest 281. Disease spatial analysis mapping, based upon geographic longitude and latitude location of herds with positive diagnostic tests, illustrate progressive disease expansion from the southern counties of Iowa, near the Missouri border, into the northern parts of Iowa. These results suggest that *A. marginale* has developed a widespread distribution across the state of Iowa.

Key words: *Anaplasma marginale*, bovine, Iowa, tick

Introduction

Anaplasma marginale, the etiological agent of bovine anaplasmosis, has been a recognized blood borne pathogen for over 100 years in the United States. This type of anaplasmosis specifically targets, infects and replicates inside the bovine erythrocyte.[1] Clinical infection occurs following splenic macrophage destruction of erythrocytes which harbor a threshold number of the organism. Depletion in erythrocyte numbers can result in anemia, hypoxia, abortion, jaundice and sudden death. Cattle that survive this acute phase, or have lower levels of initial bacteremia, may become chronic, persistent carriers.[2]

Diagnosis of the presence of *A. marginale* in the cattle herd requires consideration of the disease incubation period following the initial pathogen transfer. This incubation period can range from 7 days to greater than 8 weeks.[3] Testing methods conducted during the incubation period may result in false negatives. Economical screening and detection of subclinical persistently infected carriers involves collection of a serum sample for a competitive Enzyme-Linked Immunosorbent Assay (cELISA) antibody level. A reported percent inhibition of ≥ 30% is considered a positive result.[4] Diagnosis of acutely infected individuals may yield false negatives using only the cELISA test, as the antibody response takes time to develop. Direct organism DNA detection with the real-time polymerase chain reaction (PCR) molecular technique provides the most sensitive method for diagnosis of acutely infected individuals. A cycle threshold (Ct) value of ≤ 35 is considered a positive result (based on validation that determined the highest Ct value demonstrating 95% repeatability of the PCR target).[5] PCR is often utilized as a confirmation of the cELISA for carrier status animals, although due to the cyclical nature of persistent infection, organism DNA in whole blood samples may not always be detected.[6]

Carrier animals serve as subclinical reservoirs of the organism, and allow anaplasmosis to spread via mechanical or biological vectors to susceptible herd-mates. Mechanical vectors, such as multiple use needles and blood-sucking flies, transmit the organism via direct blood transfer from an infected animal to a naïve animal. Ticks function as the primary biological vector for anaplasma spread from persistent carriers to susceptible individuals. Ticks acquire the organism from clinically or persistently infected cattle and amplify a single organism first in their midgut epithelium, followed by a second round of amplification occurring in the salivary glands of the tick.[1-3,7,8] If or when an infected (usually adult male) tick feeds on additional cattle, it can introduce a substantial number of organisms into this next animal. Furthermore, juvenile nymph life stages have the potential to acquire the organism from carrier cattle and transmit it during molting to adult ticks. This mode of transmission is commonly known as transstadial disease transmission.[1,9]

The presence of infected carrier cattle and competent tick vectors in the local environment can indicate anaplasmosis is endemic in a region. Infected ticks can be transported via cattle and wildlife from one geographic region to another resulting in disease spread and the development of new endemic areas.[8,10,11] Historically, *A. marginale* has not been considered an endemic pathogen in cattle across the state of Iowa. However, biological vector tick species capable of anaplasmosis transmission are found across the state with the prominent vector *Dermacentor variabilis* (American Dog tick), representing > 50% of the ticks identified in Iowa.[12] The occurrence of the disease has been further influenced by the relocation of

cattle into Iowa from traditional endemic areas of the country. Additionally, the persistent drought conditions in the central and southwestern United States have led carrier animals to migrate into Iowa and other parts of the Midwest.

Acute cases of bovine anaplasmosis have been reported, with confirmatory diagnosis at the Iowa State Veterinary Diagnostic Lab (ISU VDL), from the counties along the Southern border for over 20 years. Published studies related to Iowa seroprevalence of anaplasmosis include a single feedlot arrival study evaluating overall carcass performance and a single dairy study evaluating within-herd antibody levels and corresponding milk production.[13,14] Studies depicting the current geographic distribution and potential prevalence estimates of bovine anaplasmosis in Iowa are lacking. A recent retrospective study reviewed positive post-mortem and PCR diagnostics performed at ISU VDL from 2003-2021.[15] This study determined that most cases occurred between August and November, although geographic location of these positive animals was not reported nor limited to cattle residing in Iowa.

The main goal of our study was to analyze and present the geographic distribution trends of *A. marginale* test-positive cattle in Iowa, utilizing data from ISU VDL records, covering a 5-year period from 2017 to 2022. The study aimed to assess the hypothesis of whether *A. marginale* is present throughout the entire state by examining case submissions from Iowa veterinarians and employing spatial analysis mapping.

Materials and methods

Animals and samples

This study retrospectively evaluated ISU VDL testing results for molecular and serological detection of all stages of *A. marginale* infection in cattle. Submissions were retrieved by a single reviewer, from the Laboratory Information Management Systems (LIMS) database using the keywords "anaplasmosis" and "bovine". Results included in this analysis were submitted by field veterinarians for disease diagnosis and/or screening, and limited to samples from cattle located in Iowa, with positive results for real time PCR (cycle threshold value of ≤ 35) and competitive ELISA (≥ 30% inhibition) from January 1, 2017 until January 1, 2022.[4,5]

Data analysis

Data was compiled into an Excel® spreadsheet for descriptive analysis. The state of Iowa was divided into four geographical districts (1 = Northeast/NE, 2 = Southeast/SE, 3 = Southwest/SW, 4 = Northwest/NW) using the Iowa Congressional Districts Listed by County effective since the Iowa 2012 Election (Figure 4).[16]

The number of test positive cattle in each district was determined. Positive results were further sorted by year, type of diagnostic test (PCR or cELISA), season (winter, spring, summer, fall), and submissions per farm/owner.

Data mapping

Individual animal positive tests were recorded by the farm of origin's physical geographic address. The physical address was then converted to longitude and latitude. Thirty-two individual samples that did not have a physical address identified, were not included in the data mapping and were removed from the data set. Spatial disease maps were generated by using the longitude and latitude coordinates of each farm for each year, allowing for the identification of Iowa distribution trends associated with disease spread over the 5-year period. It's important to note that while many farms had multiple sample submissions each year, they were considered only once per year in the spatial disease map analysis to avoid duplication and maintain accuracy in the geographic map assessment.

Data mapping and cluster analysis were performed in R software (R version 4.3.1) using "sf" and "splancs" R packages. [17] A space-time interaction K function (REF) was utilized with geographic locations of sites (longitude and latitude, projection in datum NAD83) and submission year. The modeling estimated the interaction between the distance and time of *A. marginale* infection in cattle. A map of Iowa was used to create a polygon delimiting the *A. marginale* infection area. A $P \leq 0.05$ was used to establish statistical significance of time-space interaction.

Results

Total test-positive cattle

A total of 5,307 diagnostic tests for *A. marginale* were performed on cattle residing in Iowa at the ISU VDL for the 5-year period from January 1, 2017-2022. The total number of positive tests was 1,063, comprised of 154 PCR molecular and 909 cELISA serological tests. Overall, 20% of the tests performed were positive for *A. marginale* with a range of 12.7% to 28.8% over the 5-year time period (Table 1).

Table 2 presents the distribution of total positive tests by geographical district. Out of the 1,063 positive tests, the northeast (282) and northwest (281) districts of Iowa had the highest numbers. The highest number of positive submissions occurred in 2018 (319), followed by 2020 (255), 2021 (207), 2017 (142), and 2019 (140). It is worth noting that all districts had a relatively similar number of positive tests, indicating that the disease is no longer confined to the southern tier of Iowa counties. The disease has a presence in all the different regions of Iowa.

Diagnostic test method types

Diagnostic tests used to identify positive cattle were PCR and cELISA. The 154 positive molecular PCR tests accounted for 14.5% of all positives, and the 909 positive serological cELISA tests accounted for the remaining 85.5% of all positive tests (Figure 1 and Table 2).

The NW district had the greatest number of positive PCR tests. In examining the submissions, it was noted that one cow-calf farm with a disease outbreak investigation in 2021 accounted for 27 (23%) of the positive tests. The NE and SE districts had the same number of cELISA positive tests, followed by the NW and SW districts (Table 2). The type of cattle operation and the age of test-positive cattle was provided by the referring veterinarian in some of the sample submission records. Cow-calf operations taking samples from adult cattle represent the majority of test positive cattle in this study data set.

Test-positive cattle by season and year

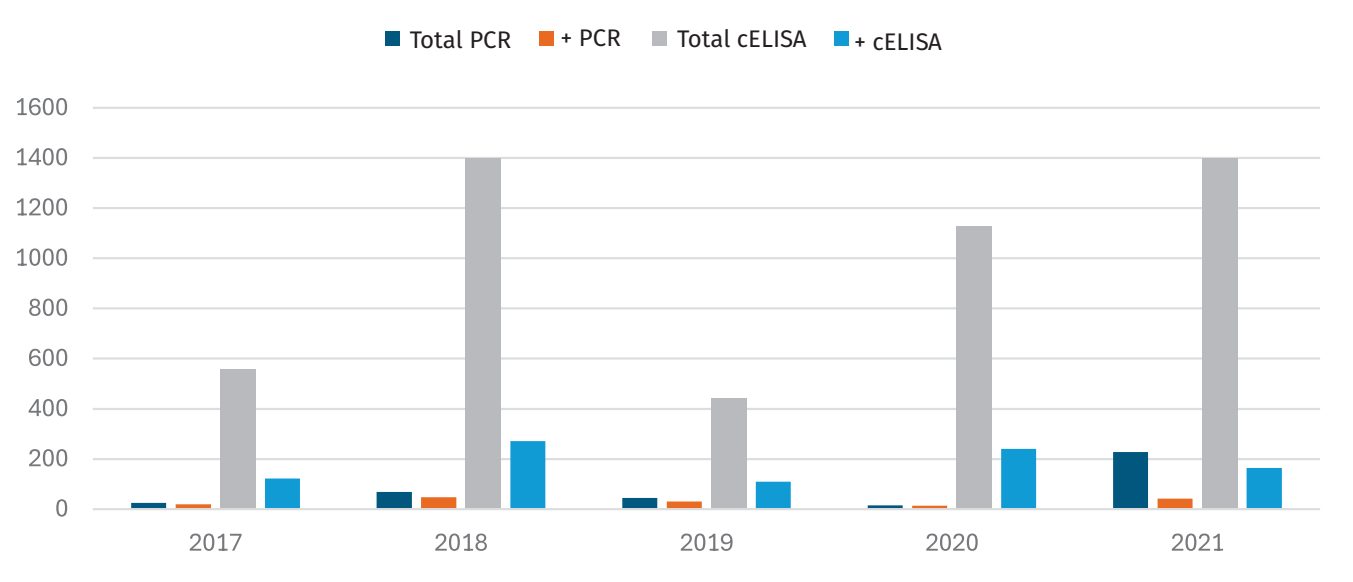
Positive test results were categorized into season of year for each of the 5 years included in the study (Figure 2). The greatest overall number of positive tests, 456 (43%), occurred in the fall months of October, November and December. The spring months of April, May and June recorded the second highest

Table 1: Bovine test submission results for *A. marginale* from ISU VDL 2017-2021.

Year	Total tests	+* Tests	Total PCR	+* PCR	Total cELISA	+* cELISA
2017	584	142 (24.3%)	25	20	559	122
2018	1467	319 (21.7%)	69	48	1398	271
2019	486	140 (28.8%)	45	30	441	110
2020	1144	255 (22.3%)	15	14	1129	241
2021	1626	207 (12.7%)	228	42	1398	165
Total tests	5307	1063 (20.0%)	382	154	4925	909

+* equals positive tests

Figure 1: Bovine test submission results for *A. marginale* performed by ISU VDL by diagnostic method each year (2017-2021).



number of positive tests with 230 (22%). Summer months of July, August and September were next at 215 (20%), followed by the winter months of January, February and March at 162 (15%). No yearly, seasonal or monthly pattern could be identified by geographical district. Instead, the districts differed widely in the number of positive tests each year and for each season and month.

Farms with test-positive cattle

The SE district had the highest number of farms submitting samples each year, followed by SW, NE and NW districts. More farms submitted samples in 2018 (68), followed by 2020 (61), 2019 (57) and equal number of farms for 2017 (55) and 2021 (55). Some farms (20 in total) repeatedly submitted additional samples over multiple years. Table 2 depicts the number of farms with positive diagnostic tests per district each year of the study. No information regarding farm size, number of animals in the herd, or the proportion of the herd sampled was available for this analysis.

Spatial analysis disease mapping

The number of farms in each county with at least one test-positive animal were plotted to provide visualization of *A. marginale* spatial analysis and potential areas of disease outbreaks. No statistical evidence of spatial clustering or outbreaks of disease were found using a space-time K function. The presence of *A. marginale* in Iowa districts and counties does not appear linked to any large clinical disease outbreaks incorporating test submissions from multiple farms in the same region. The disease maps in Figure 3, illustrate expansion of detection from the southern counties of Iowa, near the Missouri border, into the northern parts of the state. Diagnostic testing was only conducted in 93 out of the total existing 99 Iowa counties, thus documentation of a complete statewide presence could not be determined. Figure 4 provides a visual summary of the location of positive tests by county across the state of Iowa from 2017-2022. Some counties without submissions (unshaded areas) had positive tests before 2017. Additional unshaded counties have submitted positive tests since January 1, 2022. Every county in the state now borders a county with positive tests.

Table 2: Positive test distribution for *A. marginale* in each Iowa district by year, testing method utilized and farms submitting samples.

	2017	2018	2019	2020	2021	Individual total + tests		District total + tests
						PCR	cELISA	
NORTHEAST								
# + tests	62	124	36	50	10	40	242	282
# + farms	10	14	8	13	8	N/A[a]	N/A[a]	53
SOUTHEAST								
# + tests	33	49	70	70	55	35	242	277
# + farms	23	27	25	27	31	N/A[a]	N/A[a]	133
SOUTHWEST								
# + tests	35	55	24	86	23	31	192	223
# + farms	15	17	18	14	9	N/A[a]	N/A[a]	73
NORTHWEST								
# + tests	12	91	10	49	119	48	233	281
# + farms	7	10	6	7	7	N/A[a]	N/A[a]	37
Total positive								
Tests	142	319	140	255	207	154	909	1063
Farms	55	68	57	61	55	N/A[a]	N/A[a]	N/A*

N/A*: some farms had test-positive cattle multiple different years of the study.
N/A[a] : unavailable

Figure 2: *A. marginale* test positive cattle in Iowa by season and year.

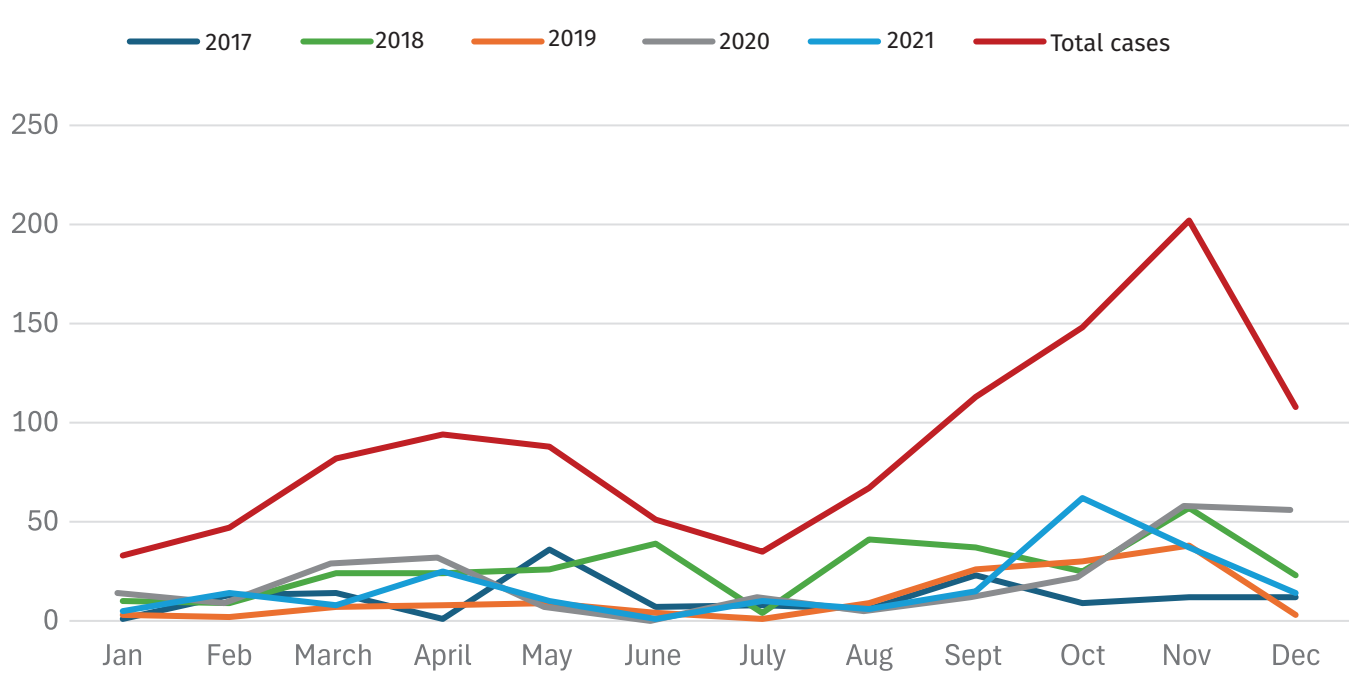


Figure 3: Spatial disease maps depicting the geographic location of farms by district each year of the study with at least one bovine *A. marginale* test positive animal in Iowa.

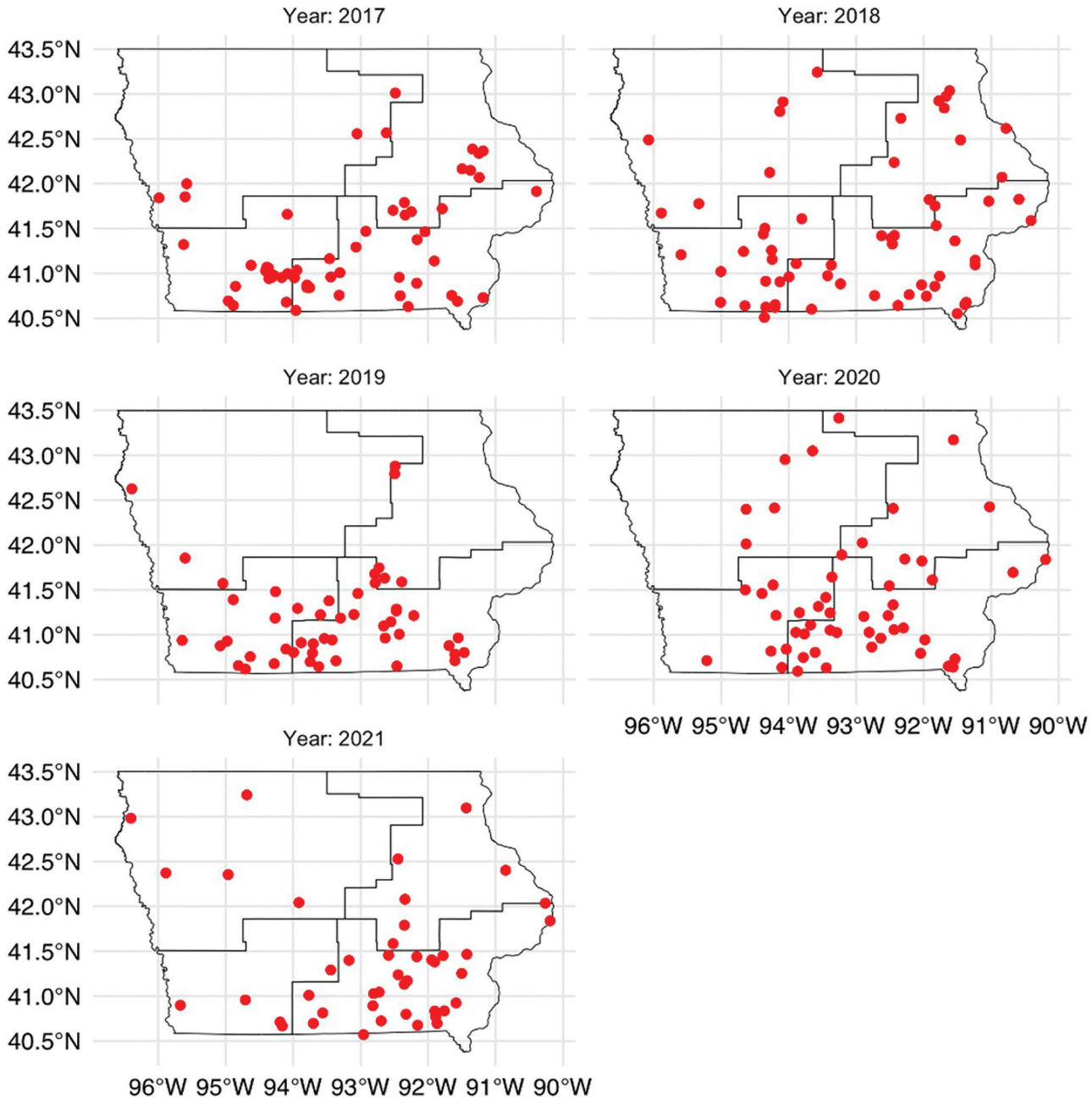
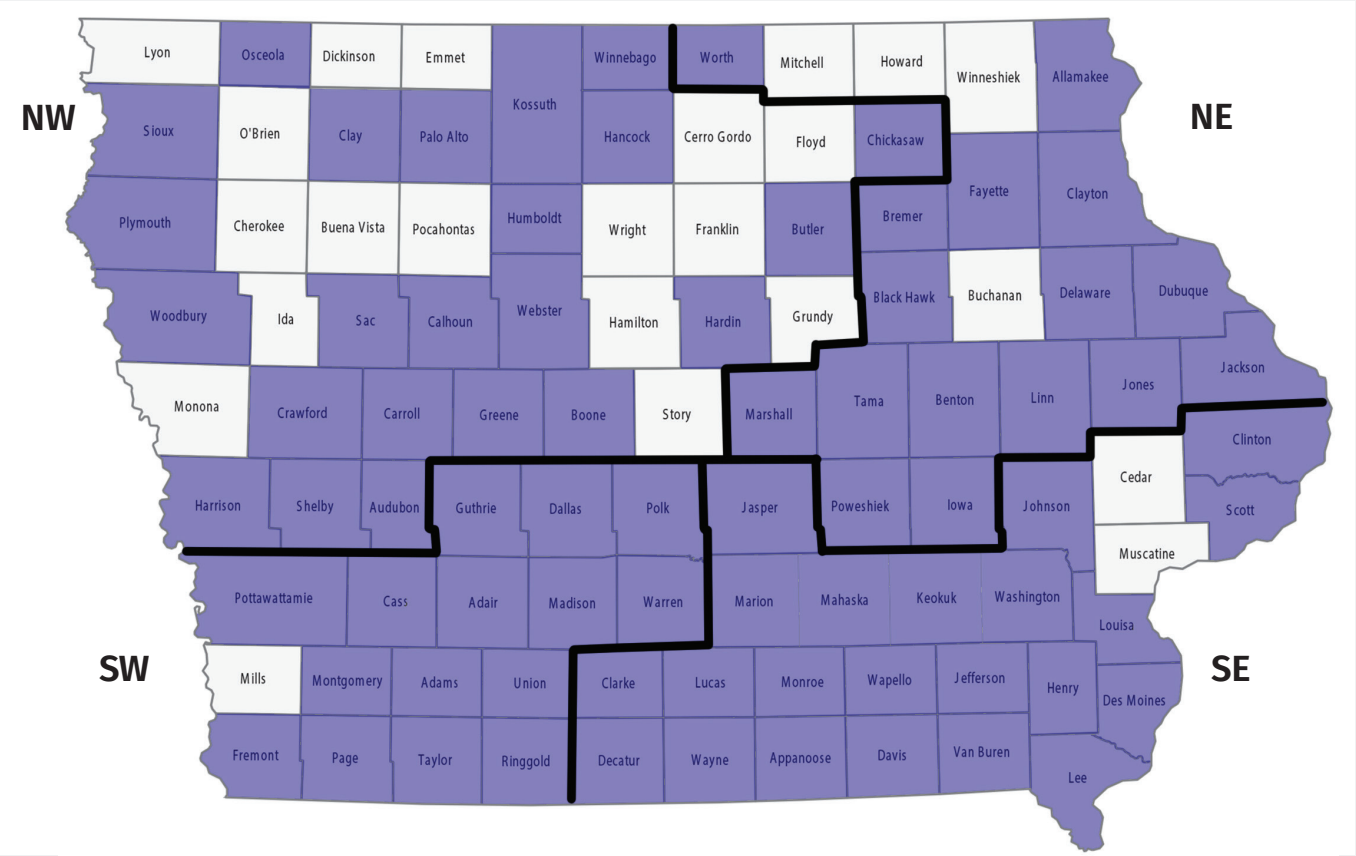


Figure 4: All Iowa counties with positive tests 2017-2022.



Discussion

This study focused upon reviewing trends and geographic distribution of ISU VDL test positive *A. marginale* cattle residing in Iowa during a 5-year time frame. This study did not attempt to calculate anaplasmosis prevalence in Iowa, as has been performed and published in other U.S. states by investigating a randomized subset of the statewide cattle population.[18-25] Unfortunately, data related to herd size and the total number of herds in each district were not available for evaluation. As a result, the total population denominator for prevalence could not be determined. This lack of information is not uncommon since submitting veterinarians rarely include details about herd size or the number of animals at risk for disease on the ISU VDL submission form. Despite this limitation, the study's findings provide valuable insights into the distribution of *A. marginale* test-positive cattle in Iowa during the specified time frame.

Statistics for cattle density in each of the 4 districts (NE, SE, SW and NW) categorized in this study was not determined. Data describing the number of feeding and cow-calf operations by county is available through the 2022 USDA NASS Census of Agriculture. The total cattle population in Iowa for January 1, 2022 was estimated at 3.85 million head. The cattle population ranged between 3.7 and 4 million head during the 5-year time period of this study.[26] In 2018, only 1,467 out of 4 million cattle in Iowa were tested at the ISU VDL, which equates to 0.04% of the population. The number of positive tests in 2018 was 319 or 0.008% of the total population. Correlation between these small percentages and the yearly incidence or overall prevalence of anaplasmosis in Iowa cannot be inferred. However, it can be concluded that anaplasmosis positive cattle reside in Iowa and that the disease has spread to all geographic parts of the state.

The number of farms that submitted at least 1 positive sample were identified by physical location as a premise infected with anaplasmosis. The spatial disease maps created of Iowa provide visual representation of the location of, both clinical and carrier cattle, for each of the 5 years evaluated. The maps confirm the widespread presence of *A. marginale* in Iowa. Cluster analysis maps representing the number of infected farms per county each year did not indicate any significant geographical areas of disease outbreak. These maps are unable to identify the intensity of anaplasmosis impact on each farm, as 1 positive test equaled a farm dot on the map each year. Some farms had more than 1 positive test, and some farms (20 in total) submitted samples for multiple years. The possibility also exists that at least some of the cattle (individual identification was not verified for each submission) were re-tested in order to evaluate the efficacy of control programs that were instituted to contain or eliminate the disease. Furthermore, some of the cattle tested could have been recent herd additions from states outside of Iowa. Finally, false negative test results could have occurred during the incubation period or early infection; along with the occurrence of potentially inconclusive positive cELISA results in the 30-40% inhibition range.[13] The true impact of anaplasmosis positive cattle in a herd depends upon the local environment and tick population, along with overall health, nutrition and management practices employed.[10]

Clusters denoting new outbreaks of *A. marginale* in localized geographic areas were not evident on the maps created or via spatial statistical analysis for this Iowa study of diagnostic lab-positive test results. Clusters were reported in the neighboring state of Kansas based upon diagnostic lab-positive test results.[27] The absence of clustering observed in this data set may be explained by the anaplasma pathogen's bloodborne transmission pattern (as opposed to direct or airborne disease contact), its prolonged incubation time, and the low but relatively constant number of test positive animals each year of the study. More widespread testing involving a defined minimum number of cattle would prove helpful in identifying an increased presence of anaplasmosis in the different geographic sections of Iowa. Once identified, areas with increasing prevalence could be further targeted for veterinary and producer education regarding disease diagnosis, monitoring of herd prevalence and evidence-based control efforts.

The direct organism DNA (PCR) and indirect antibody (cELISA) detection testing methods performed at the ISU VDL have been studied, verified and recommended for anaplasmosis diagnosis in multiple publications.[4-7] The cELISA test was requested for 92.8% of the total submissions, with the remainder utilizing the PCR test. The cELISA was presumably selected as a cost-effective screening test for many of these submissions, with a positive test interpreted as corresponding to carrier status. The reported sensitivity is 96% and specificity of 95%.[8] Determining the carrier status of new herd additions, including bulls and recipient females for embryo transfer, allows for effective herd disease management strategies.[10] Several of the farms in this dataset specialized in reproductive management programs such as bull leasing or embryo transfer. These farms submitted multiple samples as needed to screen for several different diseases simultaneously. These multiple sample submissions could be mistaken for an anaplasmosis outbreak, when in reality, they were likely part of a routine disease screening protocol. The PCR tests were typically submitted as a single sample from several farms. These individual samples probably correspond to a sick animal displaying clinical signs consistent with acute anaplasmosis infection. Bovine anaplasmosis is not directly contagious, but the presence of a clinical animal indicates a pathogen load high enough to pass the organism efficiently by the common mechanical vector of re-used needles,[28] in addition to the competent biological tick vector route. It can be speculated that if a herd has at least 1 clinically positive animal, additional persistently infected carrier animals serving as pathogen reservoirs also exist within the herd.[2]

Clinical cases of anaplasmosis are reported to be highest during the late summer and fall of the year in climates with freezing winter temperatures.[15] Tick activity is high in Iowa during the late spring and early summer when cattle are traditionally turned out onto grass.[12] The incubation period following initial exposure to infected ticks correlates with clinical cases occurring from August to November, as reported by Villar et al.[15]

In our study, the months were categorized into seasons, with the greatest number overall test-positive animals in the fall (October, November and December) followed by spring, summer and winter. Seasonality results did not show a repeatable pattern, as positive results varied by district and year of the study. The substantial elevation of positive tests in the fall of 2021 in the NW district was likely due in part to a disease investigation in one herd. As part of this investigation, PCR results on serum and whole blood were compared in order to determine the utility of serum as a diagnostic sample (Geoffroy, unpublished data). This resulted in a higher number of submissions for the NW district. These discrepancies in seasonality patterns can also be associated with the reason for test submission on individual farms. Sample collection for anaplasmosis herd screening is typically done when the herd is worked for some other management practice, such as pregnancy examination in the fall or pre-breeding vaccination in the spring. Screening prior to the beginning of the breeding season can help optimize herd culling, while screening during the winter months can address pregnant carrier cows and the potential for trans-placental transmission.[29] The ability to gather cattle for scheduled veterinary testing can also impact the season selected for diagnostic screening on larger groups of cattle.

Hanzlicek et al. reported the climate conditions of minimum land surface temperature, relative humidity and diurnal temperature range as having a greater impact than geographic region regarding anaplasmosis positive cases and overall tick survival and disease transmission in Kansas.[27] Our study did not consider factors related to climate differences between the districts. Additionally, the tick population variations between districts, changes over different years, and the level of access to the cattle population were not known or evaluated in this study. While these factors could potentially influence the distribution and spread of *A. marginale*, they were not included in the current analysis. The study focused solely on the distribution trends of *A. marginale* test-positive cattle based on ISU VDL records over a 5-year period from 2017 to 2022, without considering external factors. Further studies accounting for and limiting selection bias associated with strictly volunteer convenience sampling methods, and considering the variables of climate and tick dissemination are needed to estimate the true economic and health impacts of anaplasmosis in the Iowa cattle population.

Conclusions

This retrospective study provides evidence, based upon the widespread geographic distribution of positive anaplasmosis cattle, both as clinical and carrier status animals, along with the previous documented presence of competent tick vectors that the potential exists for *A. marginale* to become endemic across Iowa. This study does not report the actual prevalence of *A. marginale* in Iowa, but it does indicate a strong need for veterinary practitioners in Iowa to be acutely aware of the clinical signs, diagnostic testing available, treatment regimens, transmission between animals and across herds, and prevention strategies to minimize losses associated with this disease.

Author contributions

Conceptualization, LMG, TJE and MSH; methodology, LMG and TJE; software, APPS; validation, LMG and TJE; formal analysis, LMG and APPS; investigation, MBH and LMG; resources, MBH; data curation, MBH and APPS; writing – original draft preparation, LMG; writing – review and editing, TJE and MSH; visualization, LMG and APPS; supervision, TJE; project administration, LMG and TJE; funding acquisition, MSH. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

This is a review of anaplasmosis diagnostic tests in cattle at the ISU-VDL. No Institutional Review Board Statement was requested.

Data availability statement

The data are available upon request from the corresponding author.

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Conflicts of interest

The authors declare no conflict of interest

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