5-2023

Evaluation of Anaplasma marginale ELISA Positive Cattle for Co-Infection with Ehrlichia spp.

Grayson Robbins
University of Arkansas, Fayetteville

Evaluation of *Anaplasma marginale* ELISA Positive Cattle for Co-Infection with *Ehrlichia* spp.

Undergraduate Student Researcher: Grayson L. Robbins, glrobbin@uark.edu

Research Mentor: Lauren R. Rogers DVM, lrt03@uark.edu

University of Arkansas

Table of Contents

Abstract

Bovine Anaplasmosis is an infectious, hemolytic disease transmitted by the rickettsia parasite *Anaplasma marginale* in cattle. *A. marginale* parasitizes the red blood cells of an animal throughout their lifetime. The beef cattle industry is the fifth largest agricultural commodity in Arkansas, so potential economic losses due to bovine anaplasmosis is a critical issue. Previously tested beef cattle herds at the University of Arkansas' Savoy and Batesville units have tested seropositive for *A. marginale* with low percent inhibitions; however, all seropositive animals tested negative for infection on DNA analysis. The objective of this study was to determine the current infection status of individuals within the two beef cattle herds that have previously tested seropositive for *A. marginale* and to potentially identify a similar pathogenic organism that may contribute to antibody cross-reactivity. Historically, herd management recommendations have been based on serological testing; however, producers could potentially be making decisions on which cows to treat or cull based on false positive results. Whole blood samples were analyzed for active infection with *A. marginale* through quantitative Polymerase Chain Reaction (qPCR) testing and blood serum samples were analyzed for anti-*Anaplasma* antibodies through Competitive Enzyme-linked Immunosorbent Assay (c-ELISA) testing. Additionally, IDEXX 4Dx snap testing and qPCR was performed to detect *Ehrlichia* spp. and assess potential cross-reactivity of pathogens. Among the two research herds, there were 44 previously seropositive cows available for re-evaluation. 10 were seropositive for *A. marginale*, 10 were seropositive for *Ehrlichia* spp., and no cows tested positive for active infection with either rickettsial species. Of the 10 *A. marginale* seropositive cows, only 50% also showed an antibody response to *Ehrlichia* spp. The results of testing contribute valuable information to ongoing research regarding the prevalence of *A. marginale* and/or *Ehrlichia* spp. within the university's beef cattle herds.

Introduction and Literature Review

Bovine anaplasmosis has a significant impact on beef and dairy production worldwide. Zabel and Agusto (2018) estimated the impact of anaplasmosis entering a cattle herd as 3.6% reduction in successful calving, 30% increase in the cull rate, and 30% mortality in adults that show clinical signs. Marques et al. (2020) estimated the global losses due to bovine parasitic diseases at $13-18 billion per year and yearly milk losses of 90.2 liters per cow, which would lead to production losses estimated at $922 million yearly. Bovine anaplasmosis is a vector-borne, infectious disease caused by the rickettsial bacterium *Anaplasma marginale*, a pathogen that parasitizes and destroys the red blood cells in the body. The destruction of red blood cells prevents adequate oxygen transport to the body's tissues and organs, resulting in clinical signs such as severe anemia, decreased milk production, weight loss, abortion, and death (Kocan et al., 2004). Cattle are prone to contracting and spreading this disease in a herd, and while the infection is treatable, infected individuals become life-long carriers (Zabel and Agusto, 2018).

Anaplasma marginale is typically biologically transmitted via an arthropod vector, but other means of transmission include mechanical transmission via blood contaminated needles, surgical equipment, and biting flies. Biological transmission occurs via the salivary glands of ticks that have been infected by *A. marginale* in the cells of the midgut and that subsequently feed on cattle (Kocan et al., 2004). In the U.S., male *Dermacentor* ticks are demonstrated as being persistently infected and capable of transmitting *A. marginale* between cattle (Kocan et al., 2010). Once exposed, cattle also become persistently infected with *A. marginale* and serve as reservoirs of infection for naïve animals; however, clinical signs only manifest during active infection when there is a high pathogen load in the erythrocytes of the body (Kocan et al, 2004).

Therefore, the subclinical nature of bovine anaplasmosis compromises a producer's ability to accurately determine the infection status of their herd (Spare et al., 2020).

Diagnosis of persistently infected cattle requires serological and molecular testing. Competitive enzyme-linked immunosorbent assay (cELISA) is often used as a screening tool for detecting the seroprevalence of *A. marginale* in cattle herds. Molecular testing, on the other hand, is used for confirmatory diagnosis of active infections. Quantitative polymerase chain reaction (qPCR) tests whole blood for antigen presence. While qPCR assay is a more accurate indicator of *A. marginale* infection, cELISA is a practical method for diagnosing a large number of cattle. cELISA is more accessible to producers and is cost effective at $3-6 per head, while qPCR costs $25 per head (Arkansas Veterinary Diagnostic Laboratory, 2021). Cross-reactivity between species within the *Anaplasmataceae* family causes concern with serological methods, however, and can prevent accurate determination of the causative pathogen of infection in a herd (Kocan et al., 2010). Therefore, the potential cross-reactivity between genetically related species may result in imprecise results from the exclusive use of an ELISA serum test (Reinbold et al., 2010).

Ehrlichiosis is a tick-transmitted disease that affects multiple species, including cattle, and is caused by pathogenic agents within the *Ehrlichia* genus. While *A. marginale* and *Ehrlichia* spp. infect different cells in the body, identification of the pathogen affecting a herd can be complicated by seroconversion and cross-reactivity due to the close, phylogenetic relationship that their causative rickettsia bacteria share (Al-Adhami et al., 2011). The *Anaplasmataceae* family consists of the genus *Ehrlichia* in which the species naturally affecting cattle include *E. ruminantium* and *E. minasensis*; however, cattle can mount an immune response

to other *Ehrlichia* spp. that do not cause active infection, creating potential for antibody cross-reactivity (Moura de Aguiar et al., 2019).

Although the more well-known species of *Ehrlichia, E. ruminantium*, is endemic to Sub-Saharan Africa and the Caribbean islands, studies have indicated the presence of a novel species in North America. A novel strain of the *Ehrlichia* pathogen, *E. minasensis,* has been isolated in cattle located in Brazil and Canada and is considered to have a wide geographic distribution due to the variety of ticks that transmit it. Postmortem observations and genome sequencing of *E. minasensis* studied by Moura de Aguiar et. al (2019) found that *E. minasensis* most likely originated from *E. canis*, affecting canines, and experienced a host-shift through genome expansion. *E. minasensis* has been identified in several tick species within the *Rhipicephalus* and *Amblyomma* genera, including *R. sanguineus* (Moura de Aguiar et. al, 2019). *R. sanguineus*, or the brown dog tick, is distributed worldwide and is the primary biological vector of *E. canis* in dogs (Ferrolho et. al, 2016). *E. canis* infection is also called canine monocytic ehrlichiosis, referring to the pathogen's parasitism of the body's monocytes and macrophages, causing an immune-mediated platelet destruction. Additionally, exposure to *E. ewingii*, causing granulocytic ehrlichiosis in both dogs and humans, may produce an antibody response in cattle. *E. ewingii* is naturally carried by *Amblyomma americanum,* or the lone star tick, which has a high distribution in the state of Arkansas (CDC, 2022).

André et. al (2020) reported a high rate of co-infection in sampled cattle with 22% of co-infection occurring by *A. marginale* and *E. minasensis*. Cross-reactivity observed in serological testing for *A. marginale* and *Ehrlichia* spp. leads to confounding results, so differentiation should occur through PCR analysis. The study conducted by Al-Adhami et. al (2011) reported cattle infected with a novel strain of *Ehrlichia* spp. that were also seropositive for *A. marginale*, but

they suggested that false-positive serological test results can arise, decreasing the sensitivity of cELISA tests.

Since an effective vaccine for *A. marginale* has yet to be fully approved by the USDA and tetracycline drugs only eliminate active infection but not the pathogen itself, management practices are often the best control of disease that a herd owner possesses. According to Spare et al. (2020), these practices could include tick surveillance, disinfecting castration and ear notching devices, changing needles in between animals, vaccinating and de-worming, and antibody testing. However, these practices have not eliminated the spread of disease between endemic and un-endemic herds. A surveillance study conducted in the state of Arkansas indicated a possible 30% positivity rate for *Anaplasma marginale* within the Ozark Mountain region, including the University of Arkansas's research herds (Apple et al., 2019). Although both cELISA and qPCR diagnostics were run, individuals that tested positive on at least one diagnostic test were determined positive overall for *A. marginale* infection. Subsequently, cELISA and qPCR testing were conducted in 2020 to evaluate *A. marginale* whole herd prevalence among the fall-calving herds at Savoy and Batesville research units. Diagnostics indicated that 19 out of 168 cows tested from the Savoy herd and 21 out of 162 cows tested from the Batesville herd were seropositive. However, all confirmatory qPCR diagnostics run on seropositive samples from the Savoy herd were negative for antigen presence, and only three cows from the Batesville herd tested low positive on qPCR.

Research suggests that *A. marginale* causes persistent infection in which the pathogen remains in the carrier's bloodstream throughout their lifetime, so DNA-based testing should detect the antigen at any concentration in the blood. While seroprevalence demonstrates an antibody response to *A. marginale* infection, results from prior testing in 2020 suggest

inconsistencies across serological and molecular methods. Therefore, the potential for cross-reactivity of similar rickettsial pathogens, such as *Anaplasma* spp. and *Ehrlichia* spp. creates concern towards serological testing as a reliable management practice without confirmatory DNA analysis.

The purpose of this study was to re-evaluate the current *A. marginale* infection status among previously seropositive beef cattle within two University of Arkansas research herds. Additionally, our team sought to identify possible causes of false positivity on serological testing, specifically evaluating the possibility of co-infection with or previous exposure to *Ehrlichia* spp. These endeavors will aid in understanding the true presence of anaplasmosis and ehrlichiosis in these herds and may provide valuable insight into a potential cause of false positive results on cELISA.

Methods and Materials

Sampling Strategy

This quantitative study analyzed blood samples taken from the University of Arkansas's beef cattle herds in Savoy and Batesville, Arkansas. Samples were taken from any individual from the 2020 study still in inventory that previously tested positive for *Anaplasma marginale* on either cELISA or qPCR. Previously seronegative cows that had seropositive calves in 2020 were also re-evaluated. To accurately determine the infection status of the herds, three types of testing were used. Each sample was tested with qPCR analysis, cELISA analysis, and IDEXX SNAP 4Dx Plus Test to confirm or exclude the presence of *A. marginale* and/or *Ehrlichia* spp. qPCR testing was conducted in collaboration with the Reif Parasitology Lab at Kansas State University's College of Veterinary Medicine and the University of Arkansas's Veterinary

Entomology Lab. cELISA and IDEXX SNAP 4Dx testing were conducted at the University of Arkansas's Department of Animal Science.

Blood Sample Collection

All samples were collected in compliance with the University of Arkansas's IACUC protocols. With 26 and 18 individuals available for re-testing from the Savoy and Batesville herds, respectively, 44 blood samples were collected in total. Collection at the Savoy unit occurred over two, non-consecutive days in May 2022, while collection at the Batesville unit occurred over two, consecutive days in November 2022. One, 10 mL tube without additive and two, 7 mL tubes coated with K_3 EDTA were used per animal for blood collection via jugular venipuncture. Tubes coated with K_3 EDTA were inverted two to three times after collection to mix the blood with the EDTA to prevent blood clotting. Following collection, samples were kept on ice for transport and then refrigerated before being processed within a week of collection. Whole blood samples were transported to the Veterinary Entomology Lab on campus as well as shipped to the Reif Parasitology Lab at Kansas State University's College of Veterinary Medicine. Clotted blood samples, or samples collected in tubes without additive, were processed at the University of Arkansas's Department of Animal Science. These samples were centrifuged at room temperature at 3,000 rpm for 20 minutes to separate the serum from the blood cells. The serum from each sample was then pipetted into two, labeled 1.5 mL Eppendorf tubes and stored in a freezer at -28.8 ºC.

Pathogen Detection

Reif Lab PCR: Genomic DNA (gDNA) was extracted from 100 µl samples of whole blood using the Quick-gDNA™ Miniprep Kit (Zymo Research, Irvine, CA) according to

manufacturer recommendations. Final gDNA samples were eluted in 35 µl of DNA Elution Buffer and stored at -20°C. A quantitative real-time PCR (qPCR) assay targeting a portion of the single-copy, Msp5 gene was used to detect and quantify *A. marginale* in cattle blood samples. Briefly, qPCR reaction mixtures consisted of the following in a 20-µl total volume per reaction: 1X SsoAdvanced Universal SYBR Green Supermix (Bio-Rad, Hercules, CA), 0.2-µM Am msp5-F primer (5' – ATA CCT GCC TTT CCC ATT GAT GAG GTA CAT – 3'), 0.2-µM Am msp5-R (5' – AGG CGA AGA AGC AGA CAT AAA GAG CGT – 3'), and 2-µl gDNA. Amplification was performed using a CFX Connect TM Real-Time System (Bio-Rad Laboratories, Hercules, CA) with the following cycling conditions: 98°C for two minutes, 40 cycles of 98°C for 5 seconds, 60°C for 5 seconds, and 74°C for 15 seconds; and a final melting curve step (65°C to 95°C in 0.5°C increment steps at 5-sec per step). CFX Maestro Software (Bio-Rad Laboratories, Hercules, CA) was used to display results.

McDermott Lab PCR: DNA was extracted from 200 µL whole blood using the QIAmp® Blood Mini Kit (Qiagen, Hilden, Germany) according to manufacturer instructions. Eluted DNA was stored at -20°C prior to processing. Samples were screened for bacteria in the family Anaplasmataceae using qPCR to detect a 109 bp region of the 16S rRNA gene (Table 1). 5 µL of extracted DNA was combined in a 25 µL reaction with 0.5 µM each forward and reverse primers, 0.252 µM probe, 10 µL TaqMan™ Fast Advanced Master Mix (Applied Biosystems™, Waltham, MA), and ddH_2O. Reactions were run on a QuantStudio™ 3 Real-Time PCR System (Applied Biosystems™, Waltham, MA) at 50°C for 2 min, 95°C for 2 min, followed by 45 cycles of 95°C for 1 sec, 60°C for 20 sec. A synthetic dsDNA gBlock™ fragment (Integrated DNA Technologies, Coralville, IA) containing the target sequence was used as a positive control. Ct values of <31 were considered positive. Positive samples were further tested using a nested

conventional PCR assay targeting the groEL gene (Tabara et al., 2007; Takano et al., 2009). groEL PCR products were visualized on a 1% agarose gel stained with SYBR™ Safe DNA Gel Stain (Invitrogen™, Waltham, MA), and purified using the GeneJET PCR Purification Kit (Thermo Scientific™, Waltham, MA) according to manufacturer instructions. Purified products were sent for bidirectional Sanger sequencing by Eurofins Genomics (Louisville, KY). Resulting sequences were aligned and consensus sequences generated using Geneious Prime (San Diego, CA) to identify bacteria species.

Antibody Detection

ELISA testing was completed at the University of Arkansas's Department of Animal Science using the Veterinary Medical Research & Development (VMRD) *Anaplasma* Antibody Test Kit, cELISA v2 according to manufacturer recommendations (VMRD, Pullman, WA). Serum samples producing greater than or equal to 30% inhibition were indicated as seropositive, or positive for anti-*Anaplasma* antibodies.

Additionally, the IDEXX SNAP 4Dx Plus Test (IDEXX Laboratories Inc.), which is optimized for the detection of *Ehrlichia canis* and *Ehrlichia ewingii* in dogs, was completed on each serum sample according to manufacturer recommendations. In theory, this test might also detect the presence of antibodies to *Ehrlichia* spp. in cattle, even though it is not specifically labeled for cattle. Currently, there is not a commercially available *Ehrlichia* spp. antibody test for cattle. For this study's purpose, serum samples collected from cattle were tested with the SNAP 4Dx Plus Test to possibly detect the presence of *E. ewingii* and *E. canis* antibodies, which are regionally common pathogens associated with Arkansas tick populations, but are not known to cause disease cattle.

Results

Blood samples collected in this study represented cattle suspected of infection with *A. marginale* and/or *Ehrlichia* spp. located at the Savoy and Batesville cow/calf research facilities. Seropositive cows and their calves from the 2020 fall-calving season were re-evaluated for seroprevalence and antigen presence of the two, rickettsial species.

Among the 26 animals re-tested in the Savoy herd, only 9 individuals (34.62%), were still seropositive for anti-*Anaplasma* antibodies on cELISA analysis with an average percent inhibition of 37.59%. qPCR testing to detect *A. marginale* was negative for all 26 individuals, therefore molecular analysis did not observe DNA amplification and did not detect any active infections. 8 out of the 26 cows (30.77%) tested positive for *E.canis*/*E.ewingii* (*Ehrlichia* spp.) antibodies on the IDEXX SNAP 4Dx Plus Test. qPCR testing to detect an *Ehrlichia* spp. was negative for all individuals, indicating that no active infections were occurring (Table 2).

Among the 18 animals available for re-testing from the Batesville herd, only 1 cow (5.56%) was still seropositive on the *Anaplasma* cELISA test with an average percent inhibition of 35.99%. All 18 individuals tested negative on qPCR analysis for *A. marginale*, so no DNA amplification and no active infections were observed. 2 out of the 18 animals (11.11%) tested seropositive on the IDEXX SNAP 4Dx Plus Test, indicating antibody presence for *Ehrlichia* spp. qPCR testing conducted to detect DNA and active infection of Ehrlichia spp. was negative for all 18 individuals (Table 3).

Across the animals tested from both Savoy and Batesville locations, 44 samples were collected in total. Diagnostics run to detect the presence of *A. marginale* observed seropositivity in 10 out of 44 animals (22.73%) with an average percent inhibition of 37.43%, but all 44

animals tested negative for active infection on confirmatory qPCR analysis. Of the 44 animals tested with the IDEXX SNAP 4Dx Plus Test, 10 (22.73%) were seropositive for *Ehrlichia* spp. However, only 5 out of the 10 individuals (50%) that tested seropositive for *Ehrlichia* spp. were also seropositive on cELISA for *A. marginale*. Lastly, all 44 animals tested negative for the presence of antigen to *Ehrlichia* spp. on confirmatory qPCR analysis (Table 2, 3).

Conclusions and Discussion

Current literature supports that *Anaplasma marginale* is a rickettsial pathogen that causes persistent infection in bovines, specifically cattle. Following recovery from acute anaplasmosis, *A. marginale* typically remains in the animal's bloodstream throughout its lifetime. So, while the acute disease state can be resolved, apparently healthy carrier cattle serve as reservoirs of infection that are capable of spreading the pathogen to naïve members of a herd via biological and mechanical vectors.

In the present study, cattle within the University of Arkansas's research herds at Savoy and Batesville were re-evaluated for *A. marginale* antibody and antigen presence, as well as for co-infection with *Ehrlichia* spp. All seropositive cows tested in 2020 for infection with *A. marginale* were either low positive or negative on confirmatory qPCR analysis, and subsequently, all previously seropositive cows re-tested in 2022 were negative on qPCR. Of the previously seropositive cows available for re-testing at Savoy and Batesville facilities, 17 total individuals appear to have seroconverted between 2020 to 2022 to a negative status. In general, antibody titers tend to decrease over time after an active infection has been cleared by the animal's immune system, assuming no further exposure to the pathogen occurs. In 2022, approximately 18% of the individuals tested were seropositive with an average percent inhibition of 37%, which is on the low end of the positivity scale. Additionally, seropositive cows from

these herds have no history of clinical illness and no specific antibiotic treatments for anaplasmosis are known to have been rendered. Anti-*Anaplasma* antibodies detected by cELISA analysis are only produced in response to *A. marginale* infections, which is known to cause persistent infection. As a result, animals presenting an antibody response to *A. marginale* are expected to carry the pathogen in their red blood cells. However, recent qPCR testing has not detected *A. marginale* at any concentration in the blood samples from university owned cattle.

The decision to test for the seroprevalence and antigen presence of an *Ehrlichia* spp. intended to investigate the possibility of cross-reactivity between two rickettsial pathogens that belong to the same phylogenetic family. Possible cross-reactivity could serve as an explanation for positive results on *A. marginale* cELISA tests despite the lack of confirmation through qPCR testing. Serological testing using the IDEXX SNAP 4Dx Plus was conducted; however, only 17.5% of the samples tested positive for *E.canis*/*E.ewingii* and only half of the seropositive individuals were also seropositive for *A. marginale*. Additionally, qPCR testing to detect *Ehrlichia* spp. resulted in negative antigen presence for all animals in the study. Therefore, infection with an occurring *Ehrlichia* spp. was not observed, and the theory of antibody cross-reactivity was not significantly supported.

Further investigation is required to assess the reliability of cELISA as a first-line diagnostic for producers. Generally, cELISA is best utilized as a screening tool for herds suspected of exposure to *A. marginale* and is a more practical and affordable diagnostic method. However, qPCR is required to corroborate results of cELISA through detection of the antigen for *A. marginale*. The inconsistencies observed across diagnostic methods could prevent veterinarians and producers from accurately assessing the infection status of *A. marginale* in their cattle. As a result, the true prevalence of bovine anaplasmosis as it pertains to the state of

Arkansas is still unknown, and the sensitivity of diagnostics utilized to determine *A. marginale* presence across herds is significant to reporting disease. Due to the pathogenic and economic strain posed by bovine anaplasmosis, in-depth surveillance will be consequential to implementing effective control measures. As such, next steps in research should include reassessment of the University of Arkansas's research herds in their entirety as well as evaluation of herds located in regions of the state with predicted high positivity for *A.marginale* infection. Additionally, the use of diagnostic methods focused on additional pathogens may provide more specific information on the true incidence and distribution of bovine rickettsial diseases within the state.

Acknowledgements

The completion of this work was made possible by the physical assistance of many individuals. Special thanks to Dr. Kathryn Reif (Kansas State University) for her assistance in planning this project, completion of qPCR testing and in provision of testing supplies; Dr. Emily McDermott for her assistance in qPCR testing; Doug Galloway for his continued support and oversight of all ELISA testing; Bernie Krumpelman for his assistance in blood sample collections at the Savoy unit; and Dr. Shane Gadberry for is assistance in blood sample collections at the Batesville unit. Financial assistance for the purchase of testing equipment and travel was provided by both the University of Arkansas Bumpers College and Honors College undergraduate research grants and travel grant programs, respectively. Last, but certainly not least, thank you to the members of my honors thesis committee for their guidance and support in the development, writing and presentation of this research: Dr Lauren Rogers (Mentor), Dr. Jeremy Powell (Committee Member) and Dr. Brittni Littlejohn (Committee Member).

Appendix

Table 1

McDermott qPCR primers/probes

Anaplasmataceae 16S qPCR	Sequence	Reference
Forward Primer	5'-TGAAGTCGGAATCGCTAGTAATC-3'	McDermott, unpublished
Reverse Primer	5'-CAGCTTCGAGTTAAGCCAATTC-3'	McDermott, unpublished
Probe	5'-/56-FAM/ATGCCACGGTGAATACGTTCTCGG/3BHQ_1/-3'	McDermott, unpublished
groEL PCR		
Forward Primer	5'-GAAGATGC(A/T)GT(A/T)GG(A/T)TGTAC(T/G)GC-3'	Tabara et al. 2007, Takano et al. 2009
Forward Primer (nested)	5'-ATTACTCAGAGTGCTTCTCA(A/G)TG-3'	Tabara et al. 2007, Takano et al. 2009
Reverse Primer	5'-TGCATACC(A/G)TCAGT(C/T)TTTTCAAC-3'	Tabara et al. 2007, Takano et al. 2009

Table 2

cELISA, qPCR, and IDEXX SNAP 4Dx Plus Test Results at Savoy Herd

Cow #	cELISA Results	% Inhibition	qPCR *Anaplasma* Results	IDEXX 4Dx Results	qPCR *Ehrlichia* (*Anaplasmataceae*) Results
1026	Negative	1.318	Negative	**Positive**	Negative
3146	**Positive**	31.088	Negative	**Positive**	Negative
4014	Negative	-12.521	Negative	Negative	Negative
4116	**Positive**	51.194	Negative	**Positive**	Negative
C082	Negative	28.866	Negative	**Positive**	Negative
C098	Negative	-12.664	Negative	Negative	Negative
C213	Negative	-16.271	Negative	Negative	Negative
D093	Negative	26.426	Negative	Negative	Negative
E071	**Positive**	40.525	Negative	Negative	Negative
E235	**Positive**	31.723	Negative	Negative	Negative
C028	**Positive**	30.463	Negative	**Positive**	Negative
F156	Negative	12.41	Negative	Negative	Negative
2071	Negative	1.936	Negative	Negative	Negative
3135	**Positive**	38.089	Negative	**Positive**	Negative
4094	Negative	11.732	Negative	**Positive**	Negative
C070	**Positive**	46.845	Negative	**Positive**	Negative
E070	Negative	4.684	Negative	Negative	Negative
H130	Negative	29.008	Negative	Negative	Negative
H077	Negative	0.906	Negative	Negative	Negative
H148	Negative	10.866	Negative	Negative	Negative
H165	Negative	-3.395	Negative	Negative	Negative
H084	**Positive**	30.99	Negative	Negative	Negative

H065	Negative	4.546	Negative	Negative	Negative
H074	Negative	7.431	Negative	Negative	Negative
H072	Negative	-3.386	Negative	Negative	Negative
H096	**Positive**	37.422	Negative	Negative	Negative

Table 3

cELISA, qPCR, and IDEXX SNAP 4Dx Plus Test Results at Batesville Herd

Cow #	cELISA Results	% Inhibition	qPCR *Anaplasma* Results	IDEXX 4Dx Results	qPCR *Ehrlichia* (*Anaplasmataceae*) Results
5136	Negative	4.685	Negative	**Positive**	Negative
5243	Negative	-5.705	Negative	Negative	Negative
6220	Negative	4.746	Negative	Negative	Negative
5298	Negative	22.172	Negative	Negative	Negative
5068	Negative	9.036	Negative	Negative	Negative
5334	Negative	0.545	Negative	Negative	Negative
1292	Negative	21.289	Negative	Negative	Negative
4320	**Positive**	35.988	Negative	Negative	Negative
5357	Negative	-3.856	Negative	Negative	Negative
5332	Negative	14.684	Negative	Negative	Negative
7283	Negative	-1.463	Negative	Negative	Negative
4352	Negative	19.832	Negative	Negative	Negative
9225	Negative	-17.775	Negative	Negative	Negative
9234	Negative	-8.583	Negative	**Positive**	Negative
9275	Negative	-19.351	Negative	Negative	Negative
9267	Negative	-1.137	Negative	Negative	Negative
9202	Negative	-2.95	Negative	Negative	Negative

9212	Negative	-13.36	Negative	Negative	Negative

References

Al-Adhami, B., Scandrett, W. B., Lobanov, V. A., & Gajadhar, A. A. (2011). Serological cross-reactivity between anaplasma marginale and an ehrlichia species in naturally and experimentally infected cattle. *Journal of Veterinary Diagnostic Investigation*, 23(6), 1181–1188. Retrieved on October 2, 2022. https://doi.org/10.1177/1040638711425593

André, M. R., Calchi, A. C., Herrera, H. M., Zanatto, D. C., Horta, B. de, Tasso, J. B., de Souza Ramos, I. A., de Mello, V. V., & Machado, R. Z. (2020). The co-infection with Ehrlichia minasensis, Anaplasma Marginale and Anaplasma Platys is not associated with anemia in beef cattle in the Brazilian Pantanal. *Veterinary Parasitology: Regional Studies and Reports, 21*, 100437. Retrieved October 2, 2022. https://doi.org/10.1016/j.vprsr.2020.100437

Apple, G., Thomas, L.R, Ward, H. M., Reif, K.E., Anantatat, T., Flowers, M., White, A. C., & Powell, J. G. (2020). Surveillance of Anaplasma marginale in Arkansas Beef Cattle Herds. *Animal Science Undergraduate Honors Theses.* Retrieved from https://scholarworks.uark.edu/anscuht/40.

Arkansas Veterinary Diagnostic Laboratory. (2021, April). List of tests and fees – Arkansas Department of Agriculture. Retrieved January 20, 2023, from https://www.agriculture.arkansas.gov/wp-content/uploads/2021/04/ARVDL-PUBLISHED-List-of-Tests-and-Fees-asof-120920.pdf

Centers for Disease Control and Prevention. (2022, December 5). *Regions where ticks live*.

Centers for Disease Control and Prevention. Retrieved April 15, 2023, from https://www.cdc.gov/ticks/geographic_distribution.html

Ferrolho, J., Simpson, J., Hawes, P., Zweygarth, E., & Bell-Sakyi, L. (2016). Growth of ehrlichia canis, the causative agent of canine monocytic ehrlichiosis, in vector and non-vector ixodid tick cell lines. *Ticks and Tick-Borne Diseases*, 7(4), 631–637. Retrieved April 14, 2023. https://doi.org/10.1016/j.ttbdis.2016.01.013

Kocan, K. M., De La Fuente, J., Blouin, E. F., & Garcia-Garcia, J. C. (2004). *anaplasma marginale* (rickettsiales: Anaplasmataceae): Recent advances in defining host–pathogen adaptations of a tick-borne rickettsia. *Parasitology*, *129*(S1). Retrieved October 11, 2022. https://doi.org/10.1017/s0031182003004700

Kocan, K. M., de la Fuente, J., Blouin, E. F., Coetzee, J. F., & Ewing, S. A. (2010). The natural history of Anaplasma Marginale. *Veterinary Parasitology*, *167*(2-4), 95–107. Retrieved January 10, 2023. https://doi.org/10.1016/j.vetpar.2009.09.012

Marques, R., Krüger, R. F., Peterson, A. T., Melo, L. F. D., Vicenzi, N., & Jiménez-García, D. (2020). Climate change implications for the distribution of the babesiosis and anaplasmosis tick vector, Rhipicephalus (Boophilus) microplus. *Veterinary Research*, *51*(1). Retrieved October 11, 2020.https://doi.org/10.1186/s13567-020-00802-z

Moura de Aguiar, D., Pessoa Araújo Junior, J., Nakazato, L., Bard, E., Aguilar-Bultet, L., Vorimore, F., Leonidovich Popov, V., Moleta Colodel, E., & Cabezas-Cruz, A. (2019). Isolation and Characterization of a Novel Pathogenic Strain of Ehrlichia minasensis.

Microorganisms, 7(11), 528. Retrieved October 2, 2022. https://doi.org/10.3390/microorganisms7110528

Reinbold, J. B., Coetzee, J. F., Sirigireddy, K. R., & Ganta, R. R. (2010). Detection of Anaplasma marginale and A. phagocytophilum in bovine peripheral blood samples by duplex real-time reverse transcriptase PCR assay. *Journal of Clinical Microbiology, 48*(7), 2424-2432. Retrieved October 29, 2020. doi:10.1128/jcm.02405-09.

Spare, M. R., Hanzlicek, G. A., Wootten, K. L., Anderson, G. A., Thomson, D. U., Sanderson, M. W., … Raghavan, R. K. (2020). Bovine anaplasmosis herd prevalence and management practices as risk-factors associated with herd disease status. *Veterinary Parasitology: X, 3*, 100021. Retrieved on October 1, 2020. https://doi.org/10.1016/j.vpoa.2019.100021.

Tabara, K., Arai, S., Kawabuchi, T., Itagaki, A., Ishihara, C., Satoh, H., Okabe, N., & Tsuji, M. (2007). Molecular survey of *Babesia microti, Ehrlichia* species and *Candidatus* Neoehrlichia mikurensis in Wild rodents from Shimane Prefecture, Japan. *Microbiology and Immunology*, *51*(4), 359–367. https://doi.org/10.1111/j.1348-0421.2007.tb03923.x

Takano, A., Ando, S., Kishimoto, T., Fujita, H., Kadosaka, T., Nitta, Y., Kawabata, H., & Watanabe, H. (2009). Presence of a novel *Ehrlichia* sp. Inixodes granulatus found in Okinawa, Japan. *Microbiology and Immunology*, 53(2), 101–106. https://doi.org/10.1111/j.1348-0421.2008.00093.x

USDA-licensed Elisa, Celisa, and Agid Diagnostic (serology) test kit > detail. VMRD. (n.d.).

Retrieved January 19, 2023, from https://vmrd.com/test-kits/detail/anaplasma-antibody-test-kit-celisa-v2.

Zabel, T. A., & Agusto, F. B. (0AD). Transmission Dynamics of Bovine Anaplasmosis in a Cattle Herd. *Interdisciplinary Perspectives on Infectious Diseases*, *2018*, 1–16. Retrieved October 1, 2020. https://doi.org/10.1155/2018/4373981.