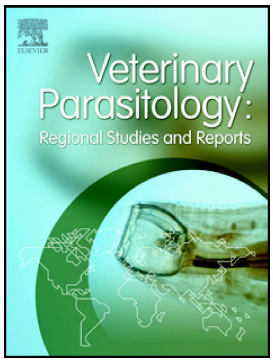

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Factors Associated with Seroprevalence of *Anaplasma marginale* in Kentucky Cattle

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Running head: Prevalence of bovine anaplasmosis in Kentucky

Abstract

Bovine anaplasmosis (BA) is tick-borne disease of cattle caused by *Anaplasma marginale* and it remains an economically important disease in the United States (U.S.). We have anecdotal information that Veterinary Feed Directive prescriptions in Kentucky (KY) are written most often for treatment and prevention of BA. However, there are no recent prevalence estimates of this disease in KY. Thus, this study was aimed at determining the seroprevalence of and factors associated with BA in KY. Data were obtained from an active slaughter survey (n = 232) performed between May and July 2013 as well as from reviewing The University of Kentucky Veterinary Diagnostic Laboratory (UKVDL) records of specimens submitted for BA testing from 2002-2012 (n = 2,573). With competitive ELISA, the apparent prevalence of BA in KY was 10.78% (95% CI: 7.41-15.42%) and 11.58% (95% CI: 10.31-12.98%) for the slaughter survey and laboratory records, respectively. Whereas the estimated true prevalence was 9.44% (95% CI: 5.65-14.48%) and 10.3% (95% CI: 8.92-11.8%), respectively. From the laboratory records, factors associated with positive BA results were age, breed, whether specimens were submitted singularly or as a group, year and quarter of the year the specimens were submitted. The odds of the outcome were 5 times as high when cattle were adults (vs juvenile) and almost 4 times as high when specimens were submitted singularly (vs group). In comparison to Holstein breed, the odds of the outcome were 3.5 and 2.5 times higher in Angus and mixed breeds, respectively. The odds of a diagnosis of BA varied in an undulating pattern by year of sample submission. When compared to 2011, the odds of a diagnosis of BA was approximately 3 times as high in 2005, 2008, and 2009 and approximately 5 times as high in 2004, 2006, and 2012. In

comparison to the duration from January to March, the odds of the outcome were almost 20 times as high from July to September but 10 times as high from October to December durations. Counties with specimen submissions for BA testing had a significantly greater cattle population and number of cattle farms than counties without specimen submissions. Future prevention and control measures for BA should target these factors and should be weighted more on counties with higher cattle population. Furthermore, current records from the UKVDL appear sufficient for the surveillance of BA in KY.

Keywords: *Anaplasma marginale*, anaplasmosis, cattle, Kentucky, prevalence

Abbreviations

AAVLD	American Association of Veterinary Laboratory Diagnosticians
BA	Bovine anaplasmosis
CFT	Complement fixation test
cELISA	Competitive Enzyme-linked immunosorbent assays
KY	State of Kentucky, USA
Se	Sensitivity
Sp	Specificity
UKVDL	University of Kentucky Veterinary Diagnostic Laboratory
US	United States of America
USDA	United States Department of Agriculture
VDL	Veterinary diagnostic laboratory
VFD	Veterinary Feed Directive

1. Introduction

Bovine anaplasmosis (BA) is caused by the rickettsial hemoparasite *Anaplasma marginale* and it is one of the most prevalent tick-transmitted disease of cattle worldwide (Dumler et al., 2001; Kocan et al., 2003; Uilenberg, 1995). This infectious but non-contagious disease is a major obstacle to profitable cattle production in many countries including the United States (U.S.) (Aubry and Geale, 2011; Decaro et al., 2008; Howden et al., 2010; Kocan et al., 2010). Infection is transmitted by biological (ticks) or mechanical vectors (biting flies), fomites (contaminated needles and surgical instruments), and less frequently transplacentally (Aubry and Geale, 2011; Kocan et al., 2010; Radostits and Done, 2007). Worldwide, approximately 20 species of ticks have been incriminated as vectors in the biological transmission of *A. marginale* (Kocan et al., 2010). However, in the U.S., interstadial transmission of *A. marginale* has been demonstrated by the 3-host ticks, *Dermacentor andersoni* and *Dermacentor variabilis* (Kocan et al., 2010).

Biological vectors are important in disease transmission because *A. marginale* can be maintained and propagated in the vector over an extended period of time, but some strains depend on mechanical transfer, which must be timely since only a fixed amount of agent is transferred (Aubry and Geale, 2011; Kocan et al., 2010; Richey and Palmer, 1990). The incubation period of infection (prepatent period) for *A. marginale* varies with the infective dose and ranges from seven to 60 days, with an average of 28 days (Kocan et al., 2010). Once an animal is infected, *A. marginale* invades and multiples within erythrocytes, which leads to, infected erythrocytes undergoing extravascular destruction and associated clinical signs. Clinical signs associated with

BA include anemia, icterus, fever, weight loss, abortions, and death (Kocan et al., 2003; Richey and Palmer, 1990).

The introduction of *A. marginale* into a naïve herd can result in a 3.6% reduction in calf crop, a 30% increase in cull rate, and a 50% mortality rate in clinically infected adult cattle (Kocan et al., 2010). The cost of a clinical case of BA in the U.S. has been conservatively 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. However, the lack of recent information regarding the prevalence of BA throughout the U.S. and its economic impact on cattle production make accurate assessment of production losses incurred by the cattle industry in the U.S. difficult, if not impossible, to estimate.

Cattle surviving BA are important in the epidemiology of the disease. Cattle that recover from acute anaplasmosis, including those treated with recommended doses of tetracycline, maintain a microscopically undetectable parasitemia for life (Aubry and Geale, 2011; Eriks et al., 1989; Kocan et al., 2010; Palmer et al., 2000; Radostits and Done, 2007; Richey and Palmer, 1990). Persistent infection is characterized by cyclic rickettsemia ranging from 10^2 to 10^7 infected erythrocytes per mL of blood that occur at approximately five-week intervals (Eriks et al., 1989; Kuttler and Simpson, 1978; Stewart et al., 1979). Although deaths may still occur, persistent infections usually confer resistance to clinical anaplasmosis (Kocan et al., 2010). Persistently infected cattle exposed to mechanical and/or biological vectors serve as reservoirs of infection to introduce *A. marginale* into populations of naïve cattle thereby leading to endemic disease stability (de Echaide et al., 1998; Futse et al., 2003; Reeves and Swift, 1977).

Strategies applied to manage BA include diagnostic testing, vector and cattle movement control, reducing iatrogenic (e.g. mechanical through contaminated needles) transmission, and administration of low doses of tetracycline antimicrobials in feed or mineral supplements (Aubry and Geale, 2011). Until the Veterinary Feed Directive (VFD) rule in 2017, BA was commonly touted reason for KY cattle to be administered oral antibiotics for long periods. Since the VFD implementation, we have anecdotal information that most recent antibiotic prescriptions in KY have been for the treatment and/or prevention of BA. 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 has been growing concern in recent years about the prevalence and economic impact of BA in cattle in KY.

Effective implementation of control strategies requires knowledge of the local or regional prevalence of BA. Estimating the seroprevalence of BA in KY is therefore a critical first step to implementing appropriate BA control programs in this state and can be a sentinel for the prevalence estimate in the region. However, the estimated prevalence of BA in KY or throughout the southeastern U.S. in the past 4 decades has not been reported in the published literature. The last reported prevalence of BA in the greater southern U.S. region occurred in the 1970's and ranged from 2% to 24% with the prevalence in KY described to be 5% (McCallon, 1973). However, complement fixation test (CFT) used to determine the prevalence has a lower Se than newer diagnostic tests for BA (Aubry and Geale, 2011; Coetzee et al., 2007). Therefore, true prevalence estimates of BA in KY and the entire region may be greater than was previously reported.

Thus, the objective of this study was to estimate the seroprevalence and risk factors associated with *A. marginale* infections in KY cattle through active purposive screening of beef cows as well as the use of previously collected laboratory records. The expected results would provide (1) farmers and policy makers the benchmark tools needed to improve the control of BA in KY, and (2) insights into the reliability of laboratory records in estimating the prevalence of BA in KY. 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

Based on an estimated prevalence of 10% (and not less than 6%), a confidence level of 95%, and a population of 995,000 beef cows from the 2012 census of the National Agricultural Statistical Service (NASS, 2014), 216 beef cows were required to estimate the prevalence of *A. marginale* in KY beef cows. This sample size was calculated using the Epi Info™ Version 7.0 software (Centers for Disease Control and Prevention, Atlanta, GA, USA). A slaughterhouse that slaughtered a significant portion of beef cattle in KY was purposively selected as a specimen collection site. This slaughterhouse, Southeastern Provision, is located in Bean Station, Tennessee. Between May and July 2013, blood specimens were collected from cull beef cows at this slaughterhouse. Only one specimen was collected from each sampled cow. For each beef cow, the individual number from a USDA-approved backtag was recorded at the time of specimen collection. Specimens were collected only from cows with backtag identifications beginning with the prefix "61", indicating KY 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, after cows were humanely stunned with a penetrating captive bolt, blood was collected (~8 mLs) from each cow in a new blood collection tube (BD Vacutainer Serum Separator; 8.5 mL). All blood specimens were transported in ice-pack containers and tested with competitive ELISA (cELISA), using the Anaplasma Antibody Test Kit (VMRD, Pullman, WA). In accordance with commercial testing guidelines, all specimens having a ≥30% inhibition were reported as serologically positive. The assay has a reported sensitivity (Se) and specificity (Sp) of 95% and 98%, respectively.

2.2. Laboratory records evaluation

The computer records of all *A. marginale* diagnostics performed between June 2002 and June 2012 were obtained from the UKVDL (Lexington, KY). Obtained records included date of specimen collection, geographic information (state, county, city, and/or zip code associated with the submission), breed and/or type of cattle, sex, age, the diagnostic assay used, and the test result. Cattle breeds with less than 100 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. Wherever age in months was captured but no categorical description was made, we updated the categorical description. Any animal < 24 months of age was classified as juvenile and anyone whose age was ≥24 was classified as adult. The BA assays used by UKVDL included CFT (discontinued after 2003) and cELISA. Data from the laboratory were harmonized (brought together from various formats and naming conventions to one cohesive data set) to facilitate analysis. All duplicate accessions, all accessions from states other

than KY, all submissions without positive or negative results were removed. Results eliminated from the data included reports that were considered “inconclusive”, “suspect”, “unable to evaluate”, and “anticomplementary”.

2.3. Analysis

In estimating the true prevalence of BA, previously described Se and Sp results of CFT and cELISA were used (Aubry and Geale, 2011; Coetzee et al., 2007). The Se and Sp results for CFT and cELISA were 26.5% and 98.0%, and 95.0% and 98.0%, respectively. True prevalence 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). Cattle population data for each county in KY were obtained from the 2012 census (NASS, 2014) to determine if cattle population and farm type (beef or dairy and size of cattle operations) differed for counties without specimen submissions, with specimen submissions having only negative results, and with specimen submissions having both negative and positive results. To display data in a visually concise format, the number of BA specimen submissions, BA positive results, and cattle population in KY, choropleth maps were created based on the slaughter survey performed between May and July 2013, state-wide diagnostic laboratory data from 2002 to 2012, and 2012 census (NASS, 2014) using ArcGIS 10.5 (ESRI, Redlands, CA).

Both univariable and multivariable logistic regression analyses were performed to test the effects of year and month of specimen submission, cattle sex, screening test type (cELISA or CFT), whether a specimen was submitted singularly or as part of a group (single or group submission),

breed and breed category (beef or dairy), and age (juvenile or adult) 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 64x (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 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., 2009a, b). The overall assessment of the final random effects and fixed effects model was done using the Bayesian Information Criteria (BIC).

3. Results

In the active BA beef cow screening, 232 beef cows were sampled from one slaughterhouse (Southeastern Provision) **(Fig. 1).** Only one specimen was collected per animal and all specimens were collected by the same individuals on 10 separate visits with a median of 23 specimens per visit. These cows originated from 18 of 120 counties in KY. 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 48 stockyards in 35 counties approved to sell cattle in KY during the survey,

and beef cows originated from 20 (41.7%) of those 48 stockyards and 18 (51.4%) of those 35 counties. The top five counties were Barren (32 samples), Montgomery (29 samples), Hardin (24 samples), Lincoln (23 samples), and Fayette (19 samples) **(Table 1).** Per county, the number of tested cattle ranged from 1 to 32 (median = 10; mean = 12.9) with 0 to 5 positives (median = 1; mean = 1.4). The county level apparent and estimated true prevalence ranged from 0 to 37.5% and from 0 to 38.2%, respectively **(Table 1).** Of the 232 sampled beef cows, 207 were negative and 25 were positive for BA. Hence the overall observed apparent prevalence of BA in KY was 10.78% (95% CI: 7.41-15.42%) while the estimated true prevalence was 9.44% (95% CI: 5.65-14.48%).

From June of 2002 to June of 2012, the UKVDL database had a total of 2,603 submissions for BA testing from 65 (54.2%) of the 120 counties in KY. However, 30 submissions (1.2%) were deleted because of either inconclusive results or submissions associated with other states. Of the balance of 2,573 submissions, 274 were positive and 2,299 were negative. With respect to the assay used, 370 (14.38%) specimens were tested with CFT while the balance of 2203 (85.62%) specimens were tested with cELISA. Of the 370 specimens tested with CFT, the overall observed apparent prevalence of BA was 5.14% (95% CI: 3.31-7.88%) and the estimated true prevalence was 12.8% (95% CI: 4.84-24.21%). However, of the 2,203 specimens tested with cELISA, the observed apparent prevalence of BA was 11.58% (95% CI: 10.31-12.98%) and the estimated true prevalence was 10.3% (95% CI: 8.92-11.8%). Of the 120 counties in KY, 64 counties had specimen submissions whereas 56 had no specimen submissions (**Table 2 and Fig. 2**). Of the 64 counties that had submissions, 34 had only negative test results (678 submissions) and 30 submitted 1,895 specimens that yielded positive (274) and negative (1,621) test results (**Fig. 2**).

There was a significant difference between counties associated with cattle tested for BA in KY and those that were not with respect to the following variables: the total cattle population ($p < 0.001$), number of beef farms ($p < 0.001$), and number of dairy farms ($p = 0.002$) **(Table 2).** However, among those counties whose cattle were tested for BA, there was no significant difference between those with positive results and those without positive results with respect to the following variables: the total cattle population ($p = 0.285$), number of beef farms ($p = 0.094$), and number of dairy farms ($p = 0.966$)**.**

At the univariable logistic regression analysis **(Table 3)**, the following variables were independently associated with the diagnosis of BA in KY cattle: cattle sex, age, type, breed, year as well as the season (quarter) of year of testing, type of assay used for testing (cELISA vs CFT), and whether specimens were submitted singularly or as a group. Females, adult cattle, beef cattle, Angus breed, cELISA assay, and singular specimen submissions were more likely to have a positive diagnosis of BA than males, juvenile cattle, dairy cattle, Holstein breed, CFT assay, and group specimen submissions, respectively. Furthermore, year as well as the season (quarter) of year of testing was associated with diagnosis of BA in KY. Although there was no yearly trend ($P = 0.0836$), diagnosis of BA was more likely in the second, third, and fourth quarters of the year when compared to the first.

Most individual factors identified from the univariable analysis remained significant in the final fitted multivariable model. The final model was estimated from a total of 1,109 cattle, of which 154 were positive for BA and included the following significant variables: age, breed, specimen submission type (single vs group), year, and quarter of year **(Table 4)**. From the final model, the

odds of the outcome were 5 times as high when cattle were adults (vs juvenile) and almost 4 times as high when specimens were submitted singularly (vs group). In comparison to Holstein breed, the odds of the outcome were 3.5 and 2.5 times higher in angus and mixed breeds, respectively. The difference in odds between other breeds and Holstein with regards to diagnosis of BA was not significant. The odds of a diagnosis of BA varied in an undulating pattern by year of sample submission. When compared to 2011, the odds of a diagnosis of BA was approximately 3 times as high in 2005, 2008, and 2009 and approximately 5 times as high in 2004, 2006, and 2012. In comparison to the duration from January to March, the odds of the outcome were almost 20 times as high from July to September but 10 times as high from October to December durations. However, the difference in odds between durations January to March and from April to June was not significant.

4. Discussion

Both slaughter survey (active surveillance) and the 10-year laboratory record evaluation (passive surveillance) methods yielded similar results. With respect to cELISA, the apparent and true seroprevalence estimates from the slaughter survey were 10.78% and 9.44 %, respectively whereas those from the laboratory records were 11.58% and 10.3%, respectively. Because the slaughterhouse data were collected between May and July and only included adults, beef cattle, and females while the laboratory records included all cattle screened throughout multiple years, the sampling frame of the slaughter survey could have contributed to an observed apparent prevalence value that is biased away from the null. Each variable in the slaughterhouse survey was demonstrated to increase the likelihood of a positive BA result at the univariable analysis of the laboratory data. In spite of this sampling advantage of the slaughter survey, the laboratory

records had a higher apparent prevalence. A possible explanation for the higher prevalence estimate from the laboratory records could be that these records include mainly specimens from animals or herds whose animals had some clinical signs of BA. Because of this possible biased selection, the prevalence estimate is likely biased away from the null. Notwithstanding, the similarity in these estimates is suggestive that laboratory records could serve as a good surveillance tool for estimating the seroprevalence of BA in KY.

In agreement with previous reports regarding BA test sensitivity (Aubry and Geale, 2011; Coetzee et al., 2007), test type used on specimens submitted to the UKVDL from 2002 to 2012 was significantly associated with BA results obtained. The apparent seroprevalence for specimens tested by cELISA and CFT was 11.58% and 5.14%, respectively. Based on the apparent seroprevalence alone, there was a 2-fold increase in prevalence of BA with an cELISA when compared to CFT. However, the estimated true seroprevalence of BA for submissions tested by cELISA and CFT was 10.3% and 12.8%, respectively. A previous study conducted 4 decades ago with CFT alone obtained a 5% apparent prevalence for BA in KY (McCallon, 1973). The apparent seroprevalence for BA in KY obtained from CFT in the present study did not appear different from that obtained previously. But the previous study did not present the estimated true prevalence. The CFT is no longer considered a reliable test for BA due to its low se property (Aubry and Geale, 2011; Coetzee et al., 2007). This could explain the reason behind discontinuing it use in BA testing at UKVDL in 2003. In spite of this limitation of CFT, the estimation of true seroprevalence would produce values that are more reliable. Therefore, it is important that prevalence studies present both the apparent and estimated true prevalence values to accommodate the deficiencies of the test parameters. Although the effect of test type was not

significant in the final model, CFT should no longer be used for BA testing. More importantly, state or regional apparent prevalence of BA obtained from specimens tested with CFT should be avoided as disease presence may be erroneously underestimated, unless the true prevalence estimate is presented as well. Much as cELISA is a better assay for BA diagnosis, certain limitations abound. These include 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). Hence, cELISA remains a valuable assay for estimating the prevalence of *A. marginale* in U.S. cattle at the moment.

Bovine anaplasmosis in the mid-western U.S., specifically Kansas, steadily increased between years 2005-2013 (Hanzlicek et al., 2016). However, the similarity between the apparent seroprevalence for BA in KY obtained from CFT in the present study (as discussed above) and that obtained previously (McCallon, 1973), could suggest that the seroprevalence may be no greater in KY presently than it was more than 4 decades ago. Although year was significantly associated with the diagnosis of BA in KY cattle over the 10-year record review period, there was no trend in this effect, suggesting that yearly spike in BA prevalence was rather sporadic than continuous.

In the present study, quarter of year of specimen submission had an effect on prevalence of BA with the lowest odds of disease observed in late winter (January to March) through late spring

(April to June). The greatest seroprevalence in the year was observed in summer (July to September) and this prevalence subsequently declined by half in fall/early winter (October to December). The seasonal variation in testing prevalence reported here is similar to that reported in Oklahoma and Louisiana (Hugh-Jones et al., 1988; Rodgers et al., 1994) and the seasonal occurrence of clinical cases in Texas (Alderink and Dietrich, 1983). Clinical outbreaks of BA occur most frequently during warm, wet seasons when vector-borne (biological and mechanical) transmission is more prevalent (Alderink and Dietrich, 1983). Naive cattle in non-endemic areas may become infected with *A. marginale* following the introduction of a carrier animal from an endemic area (Smith et al., 1989) and iatrogenic *A. marginale* infection associated with contaminated surgical equipment or hypodermic needles may give rise to clinical cases occurring outside the normal vector season (Reeves and Swift, 1977; Smith et al., 1989). Because of significance of season (quarter of year) on the diagnosis of BA as observed in the present study, we may infer that BA in KY are predominantly transmitted by vectors. However, seasonality of routine cattle husbandry that may allow mechanical transmission through iatrogenic means (e.g. vaccinations using shared hypodermic needles) cannot be ruled out.

Specimen submission type (individual submission or part of a group) was associated with positive BA result. While there were almost as many individual BA specimen submissions as there were specimens submitted as part of a group to the UKVDL from 2002 to 2012, the odds for a positive diagnosis of BA was 4 times in specimen submitted alone (as a single submission) in comparison to a specimen submitted as a group after controlling for all other factors. The reason for this observation may be simple. Possibly, a tentative diagnosis of BA was made based upon geographic location, season, signalment, and presenting clinical signs and specimens from

suspect animals were submitted to the UKVDL for further evaluation. Approximately one-fifth of the presumptive diagnosis were confirmed by serologic test. To the contrary, group submissions were more likely from entire cattle herds and not just from individual animals exhibiting clinical signs associated with BA. Thus, individuals from within a group submission could have a lower risk of being positive for BA than specimens submitted from individuals exhibiting clinical signs.

In a survey of cattle producers in Texas, bulls accounted for a disproportionate number of deaths related to BA, indicating that bulls might be more susceptible to BA than cows. This was thought to be due to a sex difference or to a lack of exposure to *A. marginale* when young, an exposure that heifers are more likely to experience (Alderink and Dietrich, 1983). In the present study, sex was not a significant factor in the positive diagnosis of BA, after controlling for other variables such as age, breed, year, and season of specimen submission. One possible explanation for these apparently contradictory findings is that the previous study sampled opinions from producers and veterinarians whereas the current study evaluated the animals and laboratory records for BA diagnosis. Alternatively, there is no sex difference in the acquisition of BA but the case fatality rate was predominantly higher in males than in females. Another possible explanation is that in general, cows remain in cattle herds for a greater duration of time than bulls and with age comes a greater risk of female cattle being exposed to potential biological or mechanical vectors of *A. marginale* and to iatrogenic exposures. The latter explanation could explain the significantly higher odds of BA found in females at the univariable analysis and not at the multivariable analysis. Because cattle age remained significant at the final model, presumably, sex confounded the relationship between cattle age and diagnosis of BA.

All cattle are susceptible to infection by *A. marginale*, but disease manifestation and detection is age-dependent. Young infected cattle rarely exhibit acute or fatal disease; however, cattle over two years of age are more prone to exhibit acute disease with mortality risks of 29% to 49% (Aubry and Geale, 2011; Jones, 1968; Kocan et al., 2003; Morley and Hugh-Jones, 1989; Rodgers et al., 1994; Rogers and Shiels, 1979), especially when older animals are stressed (Coetzee et al., 2005). It has been suggested that carrier cows in advanced pregnancy and/or lactation may relapse and develop signs of acute infection (Jones and Brock, 1966). Such events may relate to immunosuppression associated with the periparturient period in cows (Kehrli et al., 1989a, b). Regardless of the age of an animal at the time of infection, once cattle become infected with *A. marginale*, they remain persistently infected carriers for life, whether or not they develop clinical disease (Richey, 1991). Throughout the remainder of the persistently infected carrier's life, there are relatively uniform cycles over a 10- to 14-day period of increasing and decreasing numbers of circulating erythrocytes infected with *A. marginale* (Kieser et al., 1990; Viseshakul et al., 2000). Since seroprevalence for BA was higher in summer when biological and mechanical vectors are more active, it is empirical that the association between age and BA diagnosis could be a consequence of a correlation between age and exposure to biological and mechanical vectors. Thus, with increasing age, cattle are more susceptible to these vectors as well as to iatrogenic exposures. Similarly, beef cows are kept longer than dairy cows, thereby increasing their risk of being parasitized by vectors or infected through iatrogenicity. These could explain why the odds of BA diagnosis in the present study was 5 times as high in adults in comparison to juvenile cattle.

Certain breeds of cattle are more likely to have BA than others. In the present study, Angus and mixed breeds of cattle had significantly higher odds of BA in comparison to Holsteins but the association between mixed breeds and Holsteins was marginally significant. Previously, *Bos taurus* breeds (i.e., Angus, Brown Swiss, Hereford, or Holstein) have been shown to be more likely to develop acute anaplasmosis than are *Bos indicus* breeds (Zebu) or crossbreeds (Creole) (Kocan et al., 2003). These studies are in agreement that odds of BA diagnosis were greater in Angus in comparison to Holstein but differed on mixed breeds. There are other conflicting reports regarding differences in susceptibility to *A. marginale* infection between *Bos indicus* and *Bos taurus* cattle (Bock et al., 1999; Otim et al., 1980; Wilson et al., 1980). The reasons for these differences could be attributed to the differences in the type of study performed or tests used. For example, Otim et. al. and Wilson et. al. performed an experimental challenge study using CFT and immune fluorescent antibody test (Bock et al., 1999; Otim et al., 1980; Wilson et al., 1980). CFT has a lower Se in comparison to cELISA and such differences in test parameters would affect observed outcomes. Furthermore, results from experimental infected animals may differ from those from an observational study as utilized in the current study. In fact, observational studies reflect true state of the animals in their environments and therefore present a more practical approach to evaluating true risk factors of disease in a population. Breeds and/or type of cattle that spend greater time in pasture than in shelter/barns (e.g. beef compared to dairy cattle) may be at increased risk due to higher likelihood of exposure to transmission vectors (Haskell et al., 2006; Simon et al., 2016). Alternatively, the differences in geographical location and local breed susceptibility to native transmission vectors or vector preference for certain breed of cattle could play a role.

Cattle population is significantly associated with seroprevalence of BA. In the present study, counties with specimen submissions for BA testing had a significantly greater cattle population and number of cattle farms than counties without specimen submissions. Although this study did not evaluate the effect of herd size on prevalence of BA, there appear to be conflicting reports on this subject. Large cattle herds in Texas appeared to sustain BA infection more persistently than smaller herds, as the percent of herds reporting clinical cases increased as herd size increased (Alderink and Dietrich, 1983). Conversely, the seroprevalence of cattle in Louisiana to BA was independent of herd size (Hugh-Jones et al., 1988). Regardless of herd size, prevention and control measures for BA should be weighted more on counties with higher cattle population.

5. Conclusion

The estimated true seroprevalence of BA in KY was 9.44% (95% CI: 5.65-14.48%). This estimate appears similar to what the prevalence was 4 decades ago but with occasional yearly spikes in prevalence. Diagnosis of BA was significantly higher in: adults vs. juvenile cattle, Angus vs. Holsteins, individual specimens vs. specimens submitted as a group, 2012 vs. 2011, and summer vs the other seasons of the year. Future prevention and control measures for BA should target these factors and should be weighted more on counties with higher cattle population. Furthermore, current records from the UKVDL appear sufficient for the surveillance of BA in KY but all estimates should be reported after accounting for the deficiencies of the test parameters.

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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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Table and Figure Captions

Table 1: Apparent and estimated true seroprevalence of bovine anaplasmosis in Kentucky counties by slaughter survey, 2013

Table 2: Distribution of cattle and farm demographics between counties associated with cattle tested for bovine anaplasmosis in Kentucky and those that were not in the laboratory record review (2002 – 2012).

Table 3: Logistic univariable analysis for associations between various factors and bovine anaplasmosis in Kentucky cattle at the University of Kentucky Veterinary Diagnostic Laboratory, 2002 - 2012

Table 4: Final multivariable logistic regression model of factors associated with diagnosis of bovine anaplasmosis in Kentucky cattle at the University of Kentucky Veterinary Diagnostic Laboratory, 2002 - 2012

Figure Captions

Figure 1: Choropleth map of cattle population density per county in KY, number of beef cows tested, and positive results and their distribution based on prospective surveillance data for bovine anaplasmosis from May to July, 2013.

Figure 2: Choropleth map of bovine anaplasmosis specimen submissions, positive results, and cattle population per county in KY based on state-wide diagnostic laboratory data from 2002 to 2012 and National Agriculture Statistic Service data from 2012

Table 1: Apparent and estimated true seroprevalence of bovine anaplasmosis in Kentucky counties by slaughter survey, 2013

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	75,257	593	23	8 (3)	0.375 (0.137 – 0.694)	0.382 (0.098 – 0.743)
Laurel	21,148	547	8	3 (1)	0.333 (0.062 – 0.792)	0.337 (0 – 0.908)
Lincoln	64,619	638	64	23 (5)	0.217 (0.097 – 0.419)	0.212 (0.075 – 0.445)
Hardin	31,819	652	20	24 (4)	0.167 (0.067 – 0.359)	0.158 (0.042 – 0.379)
Breckinridge	39,838	619	27	10 (2)	0.2 (0.057 – 0.510)	0.194 (0.018 – 0.574)
Pulaski	70,074	1,066	33	10 (2)	0.2 (0.057 – 0.510)	0.194 (0.018 – 0.574)
Laurel	21,148	547	8	5 (1)	0.2 (-0.036 – 0.625)	0.194 (0 – 0.685)
Bourbon	55,399	398	6	7 (1)	0.143 (0.026 – 0.513)	0.132 (0 – 0.575)
Montgomery	31,235	331	5	29 (4)	0.138 (0.055 – 0.306)	0.127 (0.038 – 0.307)
Warren	49,066	710	32	8 (1)	0.125 (0.022 – 0.471)	0.113 (0 – 0.516)
Barren	85,523	1,041	63	32 (1)	0.031 (0.006 – 0.157)	0.012 (0 – 0.157)
Fayette	15,469	129	1	19 (0)	<0 (0 – 0.168)	<0 (0 – 0.167)
Taylor	25,357	425	38	16 (0)	<0 (0 – 0.194)	<0 (0 – 0.203)
Washington	37,784	572	17	14 (0)	<0 (0 – 0.215)	<0 (0 – 0.235)
Russell	45,395	335	28	10 (0)	<0 (0 – 0.276)	<0 (0 – 0.291)
Owen	20,754	296	10	28 (0)	<0 (0 – 0.121)	<0 (0 – 0.107)
Todd	21,076	150	72	25 (0)	<0 (0 – 0.133)	<0 (0 – 0.122)
Henry	22,770	360	19	19 (0)	<0 (0 – 0.168)	<0 (0 – 0.167)
Fleming	55,078	546	43	19 (0)	<0 (0 – 0.168)	<0 (0 – 0.167)

Table 2: Distribution of cattle and farm demographics between counties associated with cattle tested for bovine anaplasmosis in KY and those that were not in the laboratory record review (2002 – 2012).

Counties associated with cattle tested for bovine anaplasmosis (n = 64)							
Variable	**Minimum**	**Maximum**	**Mean**	**Lower 95% CL for mean**	**Upper 95% CL for mean**	**Standard deviation**	**Median**
Cattle population	1,612	85,523	26,704	21,749	31,658	19,834	21,496
Number of farms	68	1,244	439	377	501	248	401
Beef farms	59	1,066	368	317	420	207	333
Dairy farms	0	88	17	13	22	18	11
Counties not associated with cattle tested for bovine anaplasmosis (n = 56)							
Variable	**Minimum**	**Maximum**	**Mean**	**Lower 95% CL for mean**	**Upper 95% CL for mean**	**Standard deviation**	**Median**
Cattle population	20	49,066	10,033	7,045	13,021	11,158	6,370
Number of farms	4	828	214	163	266	193	159
Beef farms	0	710	183	138	228	168	134
Dairy farms	0	72	8	4	12	14	3

Table 3: Logistic univariable analysis for associations between various factors and bovine anaplasmosis in Kentucky cattle at the University of Kentucky Veterinary Diagnostic Laboratory, 2002 - 2012

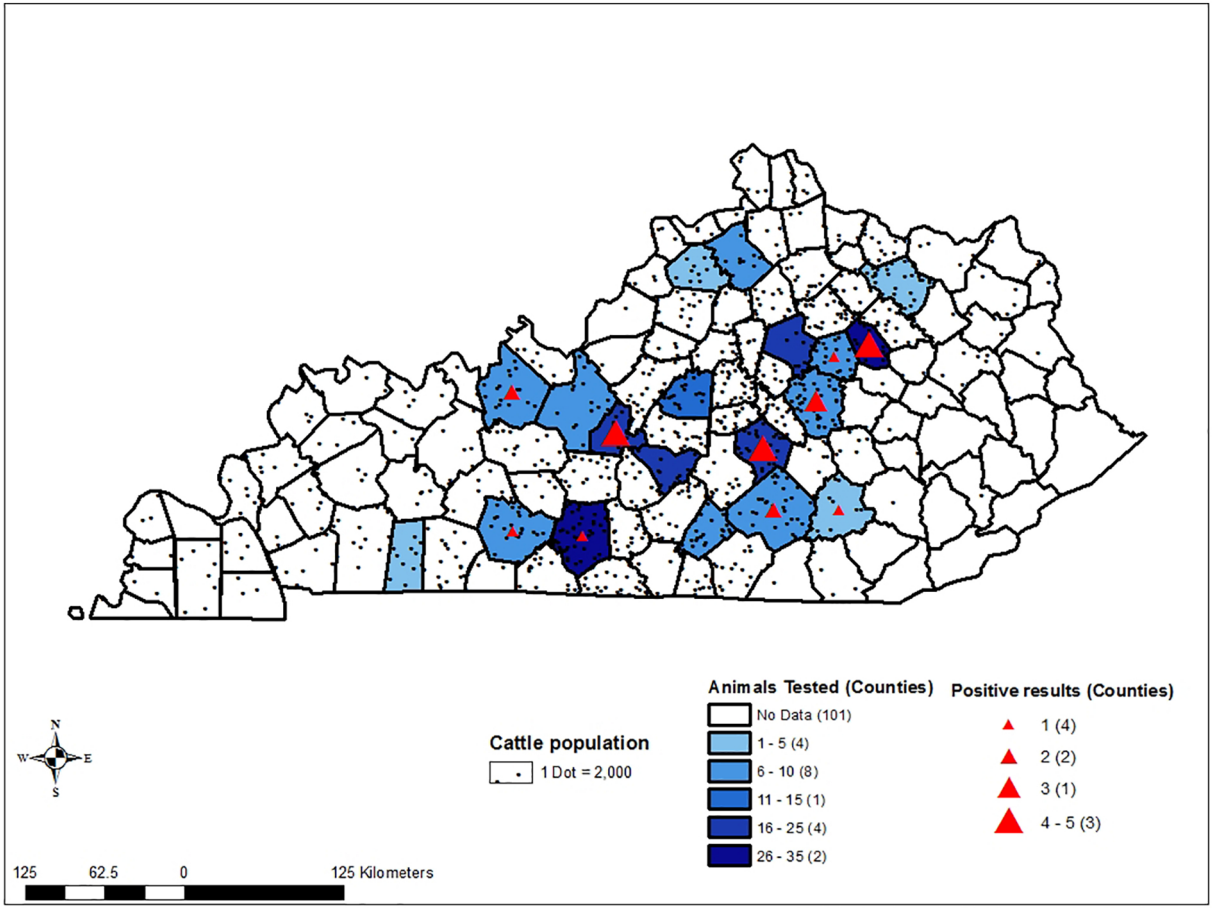
Variable	**Category**	**No. of cattle**	**OR**	**95% CI**	***P* value**
Sex	Female vs Male	1909	1.84	1.20-2.84	0.0056
Age	Adult vs Juvenile	1109	6.43	2.97-13.94	<0.0001
Cattle type	Beef vs Dairy	1586	2.96	1.90-4.62	<0.0001
Breed	Overall	2573			<0.0001
	Angus vs Holstein	837	2.88	1.76-4.72	<0.0001
	Mixed vs Holstein	1736	1.14	0.65-1.99	0.6518
	Other vs Holstein	1576	1.18	0.73-1.92	0.4966
Test type	cELISA vs CFT	2573	2.42	1.50-3.91	0.0003
Submission type	Single vs Group	2573	6.83	4.97-9.38	<0.0001
Year	Overall				<0.0001
	2002 vs 2011	496	0.78	0.41-1.51	0.4668
	2003 vs 2011	681	0.26	0.13-0.54	0.0003
	2004 vs 2011	442	2.91	1.68-5.07	0.0002
	2005 vs 2011	565	1.72	1.04-2.85	0.0347
	2006 vs 2011	457	2.10	0.20-3.70	0.0098
	2007 vs 2011	767	0.73	0.44-1.22	0.2292
	2008 vs 2011	584	0.55	0.29-1.04	0.0661
	2009 vs 2011	505	2.57	1.55-4.27	0.0003
	2010 vs 2011	552	1.35	0.79-2.30	0.2697
	2012 vs 2011	377	0.84	0.31-2.25	0.7271
Quarter of year	Overall	2573			<0.0001
	2 vs 1	1159	2.09	1.15-3.80	0.0161
	3 vs 1	1250	9.21	5.60-15.17	<0.0001
	4 vs 1	1526	5.16	3.14-8.49	<0.0001

Table 4: Final multivariable logistic regression model of factors associated with diagnosis of bovine anaplasmosis in Kentucky cattle at the University of Kentucky Veterinary Diagnostic Laboratory, 2002 - 2012

Variable	Category	OR	95% CI	*P* value
Age	Adult vs Juvenile	5.32	2.28-12.42	0.0001
Breed	Angus vs Holstein	3.53	1.57-7.96	0.0024
	Mixed vs Holstein	2.46	1.01-5.96	0.0468
	Other vs Holstein	2.19	0.97-4.95	0.0588
Submission type	Single vs Group	3.99	2.49-6.41	<0.0001
Year	2002 vs 2011	1.55	0.63-3.83	0.3373
	2003 vs 2011	2.38	0.87-6.53	0.0920
	2004 vs 2011	5.08	2.21-11.70	0.0001
	2005 vs 2011	3.32	1.21-9.11	0.0199
	2006 vs 2011	4.89	2.08-11.52	0.0003
	2007 vs 2011	2.44	0.98-6.06	0.0557
	2008 vs 2011	3.35	1.22-9.18	0.0186
	2009 vs 2011	2.78	1.07-7.28	0.0366
	2010 vs 2011	2.25	0.94-5.35	0.0675
	2012 vs 2011	5.31	1.28-22.01	0.0213
Quarter of year	2 vs 1	0.90	0.27-2.99	0.8576
	3 vs 1	19.77	8.34-46.87	<0.0001
	4 vs 1	9.96	4.18-23.72	<0.0001

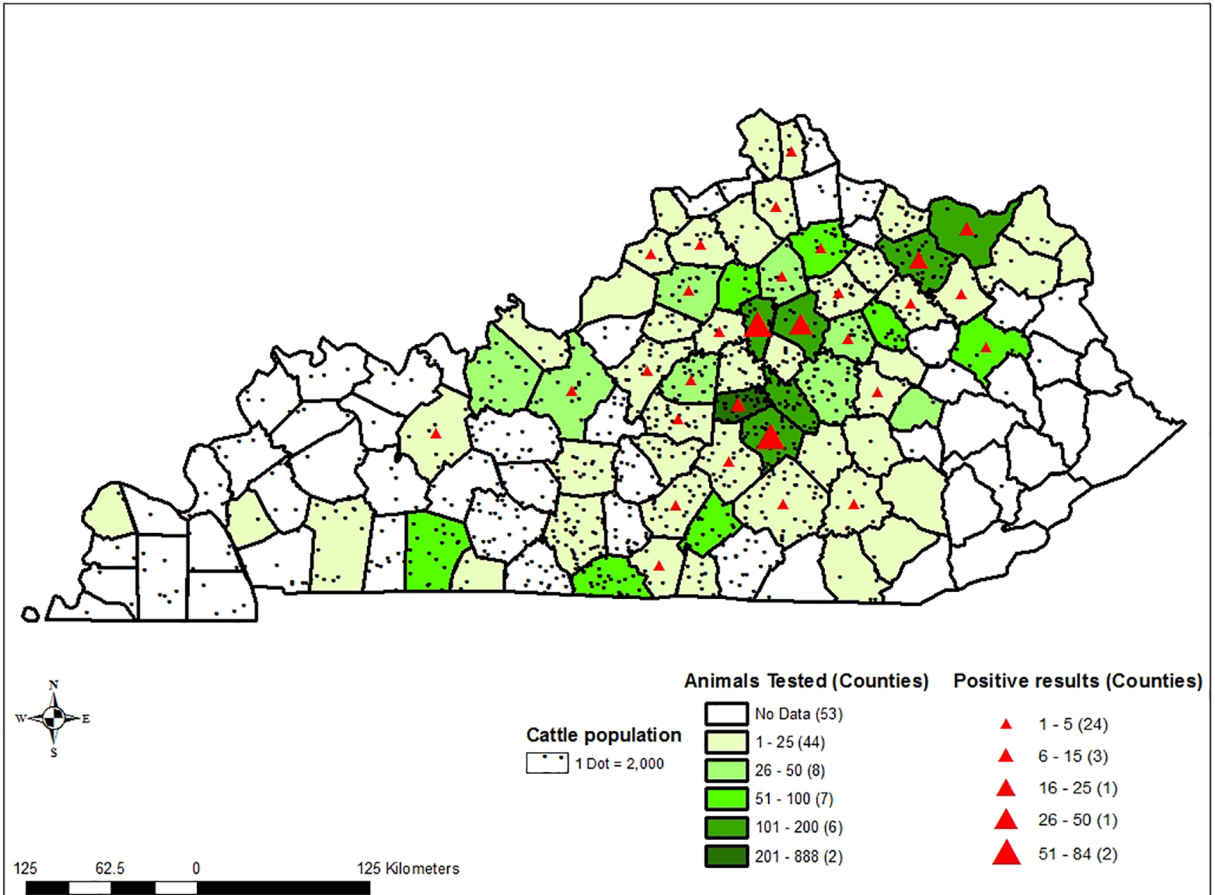
Highlights

- True prevalence of *Anaplasma marginale* in Kentucky beef cow in 2013 was 9.44% (95% CI: 5.65-14.48%)
- Prevalence was similar to estimates from 4 decades ago but with occasional yearly spikes
- Diagnosis of Bovine Anaplasmosis was higher in adults, Angus, and summer months
- Current surveillance for Bovine Anaplasmosis control in Kentucky is effective
- Future efforts should be weighted more on counties with higher cattle population



Cattle population data source: The United States Agriculture Census, 2012
Nuimber of cattle tested and positive results data source: an active survey at Southeastern Provisions slaughterhouse (Bean Station,TN) May 2013--July 2013

Figure 1


N
W
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S
Cattle population
1 Dot = 2,000
Animals Tested (Counties)
No Data (53)
1 - 25 (44)
26 - 50 (8)
51 - 100 (7)
101 - 200 (6)
201 - 888 (2)
Positive results (Counties)
1 - 5 (24)
6 - 15 (3)
16 - 25 (1)
26 - 50 (1)
51 - 84 (2)
125
62.5
0
125 Kilometers
Data Sources: University of Kentucky Veterinary Diagnostic Laboratory, 2002-2012
National Agricultural Statistics Services, 2012

Figure 2