Case-control study to identify management practices associated with morbidity or mortality due to bovine anaplasmosis in Mississippi cow-calf herds

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Abstract

Bovine anaplasmosis (BA) is a costly disease affecting the U.S. beef cattle industry. Chlortetracycline (CTC) in feed or mineral supplements is often used to control clinical signs of BA. The objective of this study was to determine if management practices, such as feeding CTC, are associated with illness or death from BA in Mississippi cow-calf herds. Case and control herds were solicited from veterinary practices across Mississippi. Cases were herds with clinical BA diagnosed by a veterinarian within the previous calendar year. Controls were herds under the care of the same practice with no clinical BA diagnosed in the previous year. Blinded interviewers conducted telephone surveys of case and control herd owners. Management and biosecurity factors were tested for association with herd status using a logistic regression generalized linear mixed model with veterinary practice as a random variable. Twenty-two case and 25 control herds from 6 veterinary practices across Mississippi were interviewed, representing 22 Mississippi counties. The average herd size was 132 breeding females for case herds, and 136 for control herds. Twenty of 22 case herds and 13 of 25 control herds fed CTC medicated mineral or feed. Providing CTC was associated with case herd status (OR = 9.2, 95% C.I. = 1.7-50.7). The association observed between case herds and feeding CTC might be because: 1) herds that had experienced previous BA morbidity and mortality subsequently began feeding CTC, or 2) some individual cattle consume enough CTC to achieve clearance of the persistent carrier state, thereby increasing risk of reinfection and clinical BA.

Key words: beef, CTC, chlortetracycline, attributable fraction, biosecurity

Introduction

Bovine anaplasmosis (BA) is caused by the obligate intraerythrocytic bacteria *Anaplasma marginale* and is considered the most important tick-borne disease affecting the United States' beef industry today.[1] Disease caused by *A. marginale* may range from subclinical persistent infection to acute, severe disease associated with significant morbidity and mortality. Clinical signs may include icterus without hemoglobinuria or hemoglobinemia, fever, lethargy, weight loss, pale mucous membranes, cerebral hypoxia leading to aggression or ataxia, abortions due to fetal hypoxia, and death.[2–5] Individuals that survive acute disease develop persistent infections that are characterized by 5 to 6-week cycles of rickettsemia.[2,6–8] Persistent infection has been reported to confer lifelong immunity to the host, while also contributing to endemic stability of the disease in populations where persistently infected individuals are frequent.[9]

Controlling BA in beef cow-calf herds can be challenging. There is currently no approved vaccine in the U.S., although a United States Department of Agriculture (USDA) conditionally licensed product is available in 28 states and Puerto Rico[a]. Recently, targeted mutagenesis methods have produced several vaccine candidates.[10]

Antimicrobials are often fed for long periods of time to control morbidity and mortality associated with BA on U.S. cow-calf operations. This use may constitute a major proportion of the antimicrobials used on these operations and raises concerns for antimicrobial stewardship. The USDA National Animal Health Monitoring System's (NAHMS) Beef 2017 study found that nationally 4.4% of all cow-calf operations, regardless of size, used chlortetracycline (CTC) in feed, mineral mixes, or mineral blocks for control of BA.[11] In the U.S., tetracyclines are the only antimicrobials labeled by the U.S. Food and Drug Administration (FDA) for treatment and control of BA. In 2020, an injectable enrofloxacin product received conditional approval for the treatment of clinical anaplasmosis in all classes of beef cattle except calves less than 2 months of age, breeding bulls, and dairy heifers less than 20 months of age[b]. Tetracycline antimicrobials represented the largest volume of sales of medically important antimicrobials used in food-producing animals in 2021.[12] In-feed use represented the most common route of administration for tetracyclines across all food-producing species, with cattle contributing 43% of the total tetracycline sales.[12] The 2 major forms of tetracyclines used in the U.S. beef industry are: 1) CTC used as a feed additive, and 2) injectable oxytetracycline. In the U.S., CTC is approved for in-feed use in beef cattle for the following reasons: treatment of bacterial enteritis and bacterial pneumonia, control of bacterial pneumonia, reduction of the incidence of liver abscesses, and control of active anaplasmosis.[13] Historically, CTC-containing mineral supplements fed free-choice to cattle during the vector season have been used in endemic or emerging infection areas in an attempt to control clinical signs associated with BA. Problems with this method of BA control include: 1) cattle may not voluntarily consume adequate amounts of CTC-containing mineral on a daily basis to maintain sufficient plasma CTC concentrations for prevention of clinical signs, 2) some cattle in endemically-stable herds may consume enough CTC-containing mineral to achieve clearance of the persistently infected carrier state, thereby making themselves susceptible to re-infection, and 3) chronic exposure of *A. marginale* and other bovine microflora to CTC may contribute to antimicrobial resistance.[4,14] Furthermore, the Veterinary Feed Directive (VFD) guidelines prohibit any extra-label use of antimicrobials in feed, including Type C medicated feeds containing CTC.[15] Granular mineral supplements containing CTC are commonly labeled to be hand fed daily, and if these products are fed in a free-choice manner, this is an illegal use.

The effect of feeding CTC-containing mineral free-choice on BA morbidity and mortality is not well understood. When offered free-choice, some persistently infected cows may consume enough CTC-containing mineral to achieve chemosterilization. Determining if feeding CTC-containing mineral free-choice is associated with the risk of BA may improve methods of BA control in cow-calf herds, as well as the stewardship of medically important antimicrobials. Therefore, the objective of this study was to determine if morbidity and mortality due to BA in beef cow-calf herds in Mississippi was associated with feeding CTC-containing mineral, or other biosecurity and vector control management practices.

Materials and methods

Study design

This study was designed as an N:M matched case-control study with veterinary practice used as the matching variable. The definition of a case herd was any beef cow-calf herd in Mississippi with morbidity or mortality in the previous calendar year due to BA that was diagnosed by a veterinarian. Diagnosis could be achieved through diagnostic testing, such as demonstrating the organism on a blood smear from an animal with clinical signs, through necropsy findings, or through veterinary examination of an animal with clinical signs. The definition of a control herd was any beef cow-calf herd in Mississippi under the care of the same practice without known morbidity or mortality due to BA in the previous year. Additionally, control herds could not be herds where morbidity or mortality due to BA was suspected in the past year but was undiagnosed. The study was submitted to the Mississippi State University Institutional Review Board (MSU-IRB) for the Protection of Humans Subjects for assessment. The study was deemed to be "Not Human Subjects Research" by the MSU-IRB, therefore exempting the study from the need for IRB approval.

Identification of case and control herds

Identification of case and control herds took place from December 2020 through February 2021. Veterinarians engaged in food animal practice across Mississippi were sent a letter of introduction to the study. The letter introduced the purpose of the study, and asked veterinarians to assist investigators in identifying beef cow-calf herds under their care that could serve as case herds. Veterinarians were provided with the definition of both case and control herds and asked to submit herds for participation in the study. Many veterinarians who received the letter of introduction were also contacted by phone, and investigators made visits to veterinary practices across the state when case herds were submitted by veterinarians to the study. Following submission of case herds for inclusion in the study, veterinarians were asked to provide a list of client herds that met the definition of a control herd under the care of the same practice. Up to 2 control herds were randomly selected from this list for each case within each practice using a random number generator[c]. Investigators asked the veterinarian to contact the case and control herd owners or managers and inquire about their interest in being included in the study, prior to the owner or manager being contacted by investigators. Once the veterinarian had secured permission from their clients to be contacted by investigators, each case or control herd owner or manager was contacted by the investigators to explain the purpose of the study, confirm case or control herd status, determine herd size, and schedule a time for a blinded interviewer to contact the producer and administer the telephone survey. Herd size was defined as the number of females in the herd that were 2 years of age or older, including heifers who had calved. Herd status was confirmed by ensuring that no morbidity or mortality suspicious of BA had occurred that the herd veterinarian was unaware of, and to ensure that no other veterinarian had diagnosed BA in the herd within the previous year.

Telephone interviews

All interviews took place from March-June 2021. Two interviewers blinded to case or control herd status conducted telephone surveys of herd owners or managers. Interviewers were trained and provided an interview transcript by investigators. Interviewees were informed that their participation in the study was voluntary, and that they could choose to opt-out of individual questions or conclude the interview at any point. At the conclusion of the interview, interviewees were provided with contact information for investigators. The questionnaire consisted of 23 yes/no, multiple choice, and short answer questions covering the following topics: 1) herd demographics, 2) herd biosecurity, 3) external/internal parasite control, and 4) methods of BA control. The questionnaire is available upon request to the corresponding author.

Sample size calculations

Data from the Mississippi State University College of Veterinary Medicine (MSU-CVM) Diagnostic Laboratory and the Mississippi Veterinary Research and Diagnostic Laboratory (MVRDL) indicated that 221 diagnoses of BA were made from a total of 529 tests performed in 2019. This number included all diagnostic tests used to diagnose BA (i.e., competitive enzyme-linked immunosorbent assay, polymerase chain reaction, necropsy findings, etc.) performed at both the MSU-CVM and the MVRDL. Evaluation of records determined that these 221 cases represented approximately 60 distinct herds. Using these data, investigators estimated that approximately 30 case herds could be solicited from veterinarians across the state. Sample size calculations were performed as described by Dupont (1988) using the following assumptions: type I error probability of 0.05% (alpha = 0.05), power of 80%, correlation coefficient of 0.2 between case herds and matched control herds, and an estimated prevalence of exposure (e.g., feeding CTC-containing mineral) in control herds of 50%.[16] Results indicated that 32 case herds with 2 control herds per case from the same practice would allow investigators to detect an odds ratio of 4.0 as a measure of association between any exposure (e.g., feeding CTC-containing mineral) and being a case herd.

Statistical analysis

Results of telephone surveys were collated into spreadsheet software[c], and descriptive statistics were performed. Inferential statistics were performed using statistical software[d], where the outcome of interest was probability of being a BA case herd. This study was designed to utilize veterinary practice as an N:M cluster-matched variable for logistical reasons because veterinarians served as a convenient source for identifying case and control herds.

Case herd status was modeled in a logistic regression generalized linear mixed model (GLMM) using PROC GLIMMIX. Veterinary practice was considered a random variable in all models to account for clustered sampling. Explanatory variables tested for association with case herd status can be found in Table 1. Following assembly of univariable models, manual forward variable selection was used to assemble multivariable models. Type 3 *P*-values and Akaike's Information Criterion (AIC) were used to determine variable inclusion and exclusion within the model. Statistical significance for all steps in the model building process was set a priori at an alpha of 0.05.

When a disease or condition such as BA occurs infrequently in a population, the odds ratio (OR) can be used as an approximate relative risk (RR).[17] The population attributable fraction (AF_p) was calculated for statistically significant explanatory variables using the following formula:

$$AF_p = P_e \ (OR - 1) \ / \ [(P_e \ (OR - 1) + 1]$$

In this formula, P_e represents the proportion of CTC-exposed herds in the population. This value was estimated by 13 control herds feeding any CTC-medicated feed or mineral divided by 25 total control herds (Table 1). The odds ratio used in the above formula estimated the relative risk from the model-adjusted OR.[18]

Results

Twenty-two case herds and 25 control herds under the care of 6 different veterinary practices across the state of Mississippi were enrolled in the study. These herds were located in 22 Mississippi counties. Figure 1 displays the distribution of participant herds by county in Mississippi. The mean case herd size was 132 breeding females, while the mean control herd size was 136 mature cows. The 22 case herds were made up of 4 seedstock herds, 14 commercial herds, and 4 herds that were both seedstock and commercial. The 25 control herds were made up of 3 seedstock herds, 19 commercial herds, and 3 herds that were both seedstock and commercial.

Table 1 displays management and biosecurity/biocontainment, as well as vector and BA control univariable model results where the outcome is modelled as the probability of being a case herd. The explanatory variable "use of any CTC-medicated feed or mineral" produced the only statistically significant model (OR = 9.2, 95% C.I. = 1.7-50.7), with case herds having greater odds of feeding any CTC-medicated feed or mineral compared to control herds. No statistically significant multivariable models were identified. The explanatory variable of whether or not the respondent used dust bags for fly control could not be tested because no control herds used dust bags for fly control. The population attributable fraction for feeding CTC was calculated to be 0.81, or 81%, using a proportion of exposed herds of 0.52 and an estimated RR from the OR of 9.2 (Table 1).

Discussion

This study identified a positive association between BA case status and feeding CTC. A 2020 cross sectional study by Spare, et al. also found use of CTC as a risk factor for being a BA case herd.[19] In the present study, the association observed between CTC use and herd status may have been present due to: 1) producers feeding CTC in mineral or feed in response to previous BA morbidity or mortality in their herds, or 2) feeding CTC in mineral or feed increased a herd's risk for experiencing BA morbidity and mortality. Presumably, the latter would happen if cattle consumed an adequate amount of CTC to clear the persistently infected carrier state, putting the animal at risk for reinfection and acute clinical disease in an endemic herd. Because the timing of CTC use relative to an initial BA diagnosis is not known, it cannot be determined if the use of CTC in feed or mineral was in response to cases of BA in the herd or if CTC use led to greater risk of morbidity and mortality in the herd. If the latter is true, and feeding CTC increases the risk of clinical BA, then discontinuing the practice of feeding CTC in some herds may have a sizable impact on the number of case herds. The AF_p of 81% indicates that feeding CTC could be responsible for as much as 81% of case herds in Mississippi and other southeastern states. Investigators empirically believe that CTC use in Mississippi is similar to that in other southeastern states. Although the NAHMS Beef 2017 study reported 3.3% of operations in the east region used chlortetracycline in feed, mineral mixes, or mineral blocks to control anaplasmosis, 70.8% used free choice loose mineral and 11.2% used a medicated mineral block as the method of administration.[11] Supplying CTC in a free-choice manner precludes any control over individual animal consumption, leading to the possibility of over-consumption by some individuals.

Previous studies have demonstrated cattle can be cleared of the *A. marginale* persistently infected carrier state by hand-feeding CTC for extended periods of time.[20,21] However, these studies either 1) used CTC doses that exceeded the FDA-approved dosing regimen of 1.1 mg/kg body weight per day, a practice that is now illegal under the current VFD regulations, or 2) relied on diagnostic testing methods that lacked the sensitivity to accurately detect subclinical, persistently infected individuals. A recent study demonstrated that the FDA-approved CTC dose of 1.1 mg/kg body weight hand fed to animals individually for 60 consecutive days failed to consistently clear the subclinical, persistently infected *A. marginale* carrier state.[22] These findings, however, do not preclude that some persistently infected cattle in a herd might clear infection and become susceptible to reinfection and potentially severe disease. Studies have shown that large variations exist in the individual daily consumption of mineral when offered free-choice to cattle in a pasture setting.[23,24] Currently, there are no limits on the duration of use for CTC-containing feed products fed for anaplasmosis control, providing that a valid VFD is maintained by the producer.[25] Data from a survey of Kansas beef producers showed that of those producers who fed CTC-containing feed products for anaplasmosis control, 76.1% fed them year-round.[19] We speculate that when CTC-containing feed or mineral is fed for extended periods of time in a free-choice manner, some individuals may consume higher than anticipated doses of CTC. This consumption may increase the probability of clearing the *A. marginale* persistent carrier state and either making that individual newly susceptible or disrupting the endemic stability of the herd, although data demonstrating this phenomenon is lacking. Furthermore, prolonged offering of FDA-approved CTC-containing mineral supplements labeled for free-choice feeding has been demonstrated to change the antimicrobial resistance profile of enteric organisms such as *Escherichia coli* in cattle.[26]

Concerns for the development of resistance of *A. marginale* to CTC exist as well, although, to the knowledge of investigators, there are currently no studies that demonstrate this occurring. Therefore, the practice of providing cattle on pasture with free-choice access to CTC-containing feed or mineral may unintentionally contribute to outbreaks of BA morbidity and mortality, as well as hasten the development of antimicrobial resistance in both commensal and pathogenic bacterial species.

Sample size may have limited the power of the study to detect associations between other risk factors and case or control herd status. No vector control measures were found to be associated with BA herd status in the present study, whereas a previous study in Kansas found the use of insecticide ear tags and the use of pour-on dewormers to be positively associated with BA herd infection status.[19] There are many reasons why risk factors identified in different observational studies may vary (e.g., study design, observing spurious results, the presence of multiple component causes, etc.). Although not statistically significant, the use of insecticide ear tags for fly control was numerically protective in the present study. Producers who have previously experienced morbidity or mortality due to BA may be more likely to employ vector control measures such as insecticide ear tags to control transmission of *A. marginale*.

It was interesting to note that investigators experienced difficulties recruiting case and control herds from Mississippi veterinarians for study participation. Investigators found that many veterinarians believed most, if not all, of their clients had at some point experienced morbidity or mortality in their cow-calf herd from BA, although these events had not been definitively diagnosed. This made it difficult for veterinarians to produce a list of client herds that they did not suspect had experienced morbidity and mortality due to BA from which controls could be randomly selected. In many instances, cattle were treated with CTC-containing feed or mineral empirically when either the producer or veterinarian believed BA was responsible for morbidity or mortality, but when a definitive diagnosis had not been made. Reasons why definitive diagnoses were not made may be 1) a general unwillingness of the producer to pay for veterinary investigation or necropsy, and 2) the tendency of deaths due to BA to occur at times of the year when weather conditions accelerate carcass decomposition (i.e., summer and fall), limiting the period of time for which deaths can be discovered before the state of carcass decay precludes diagnostic investigation. In still other situations, serologic evidence of *A. marginale* infection had been discovered in herds, leading the producer to believe that any morbidity or mortality in the herd was due to BA, even in the absence of clinical signs, other laboratory diagnostics, necropsy lesions consistent with BA, or other sufficient evidence implicating *A. marginale* as the cause of morbidity and mortality. In summary, investigators found that the definitive diagnoses of bovine anaplasmosis as the cause of morbidity and mortality, meeting our case definition, was relatively rare and empirical treatment of herds based on anecdotal evidence was common. When a definitive diagnosis was present, or when producers reported they had experienced no morbidity or mortality, investigators could be confident in case and control herd status, respectively. However, we found many cow-calf herds had experienced morbidity or mortality with no diagnostic evidence, only anecdotal, implicating bovine anaplasmosis as the cause. These herds could not be accurately classified as case or control and increased the risk of differential misclassification bias in the study (i.e., producers anecdotally believing that morbidity and mortality in their herds was due to BA); therefore, these herds were excluded from the study.

Chlortetracycline has long been used for the purpose of controlling the detrimental effects of BA in beef cow-calf herds. In this study, BA case herd status was positively associated with supplementing feed or mineral with CTC. Although these results describe Mississippi herds, the CTC-feeding practices of cow-calf producers in Mississippi are likely similar to those of cow-calf producers in other southeastern states. Therefore, these findings likely apply to cow-calf herds in other southeastern states where management practices, environment, and BA risk are similar to those in Mississippi. Because these findings may have important implications for management and control of BA on cow-calf operations, as well as antimicrobial resistance, further investigation is warranted to determine if using CTC for BA control represents good antimicrobial stewardship.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgments

This work is supported by funding from the Mississippi State University College of Veterinary Medicine House Officer Grant Program, as well as the Mikell and Mary Cheek Hall Davis Endowment for Beef Cattle Health and Reproduction. This work is a contribution of the Beef Cattle Population Health and Reproduction Program at Mississippi State University. Thank you to the veterinarians in Mississippi who assisted in identifying case and control herds, as well as Harley Chase and Breanne Keowen for conducting telephone interviews.

Endnotes

[a] Anaplasmosis Vaccine, University Products, L.L.C., Baton Rouge, LA

[b] Baytril 100-CA1, Elanco US, Greenfield, IN

[c] Microsoft Excel, Microsoft Corporation, Redmond, WA

[d] SAS for Windows v9.4, SAS Institute, Inc., Cary, NC

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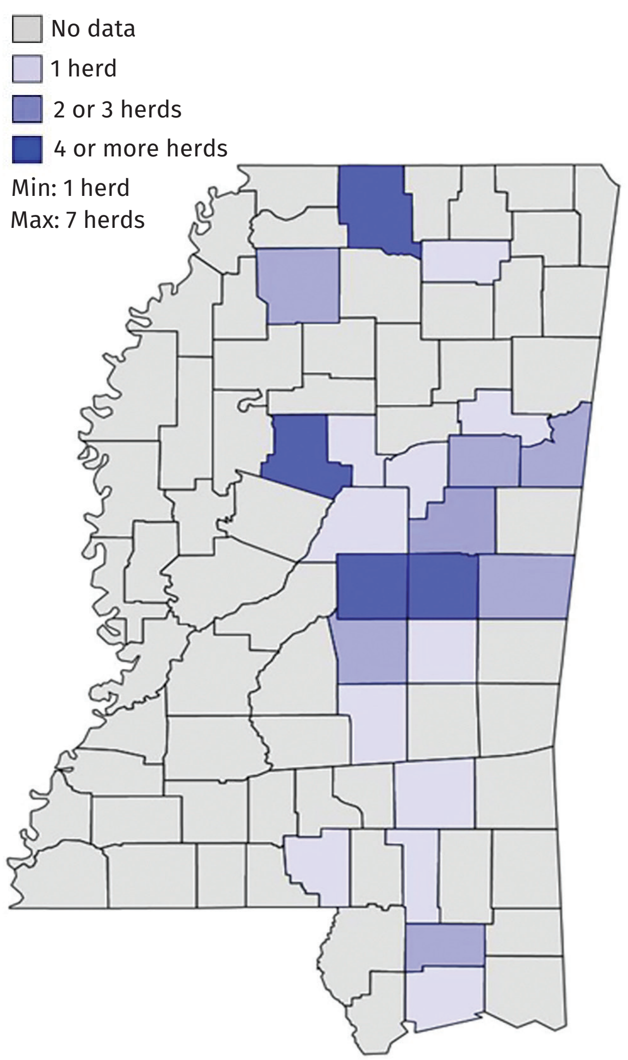
Table 1: Univariable model results for management and biosecurity/biocontainment characteristics of 47 beef cow-calf herds, 22 case herds and 25 control herds, located in Mississippi. Data was collected from March-June 2021. Outcome modeled as the probability of being a case herd.

Variable	Case herds	Control herds	Odds ratio	95% C.I.		*P*-value
Herd size (n = 43)						
200 head	4	4	0.9	0.1	5.2	0.7223
51-200 head	8	12	0.6	0.1	2.4	
≤ 50 head	8	7	1.0	Ref.		
Operation type (n = 47)						
Seedstock	8	6	1.8	0.5	6.7	0.3632
Commercial	14	19	1.0	Ref.		
Calve in spring (Mar., April, May) (n = 47)						
Yes	14	19	0.55	0.15	2.0	0.3632
No	8	6	1.0	Ref.		
Calve in summer (June, July, Aug.) (n = 47)						
Yes	5	3	2.2	0.4	10.8	0.3415
No	17	22	1.0	Ref.		
Calve in fall (Sept., Oct., Nov.) (n = 47)						
Yes	16	18	1.0	0.3	3.9	0.9559
No	6	7	1.0	Ref.		
Calve in winter (Dec., Jan., Feb.) (n = 47)						
Yes	15	17	1.0	0.3	3.6	0.9894
No	7	8	1.0	Ref.		
Fence-line contact with neighboring cattle (n = 47)						
Yes	9	14	0.5	0.16	1.8	0.3098
No	13	11	1.0	Ref.		
Approximate distance to neighboring cattle, fence line to fence line (n = 28)						
> 2 miles	3	3	1.2	0.1	9.1	0.9785
1-2 miles	6	7	0.9	0.2	5.7	
< 1 mile	4	5	1.0	Ref.		
New introductions into the herd in the previous year (n = 47)						
Yes	17	17	1.6	0.42	6.14	0.4840
No	5	8	1.0	Ref.		
# of processing events in previous year (n = 47)						
> 2 events	4	5	0.9	0.2	4.0	0.8752
≤ 2 events	18	20	1.0	Ref.		
# of injections per processing event (n = 45)						
> 3 injections	6	5	1.4	0.33	5.5	0.6689
≤ 3 injections	16	18	1.0	Ref.		
# of injections given before changing needles (n = 31)						
> 10 injections	4	3	1.1	0.1	8.3	0.9898
6-10 injections	7	6	1.0	0.2	5.3	
1-5 injections	6	5	1.0	Ref.		

Table 1 (Cont'd)

Regularly observe ticks on cattle (n = 47)						
Yes	11	14	0.8	0.24	2.6	0.6832
No	11	11	1.0	Ref.		
Use insecticide fly tags within the previous year (n = 47)						
Yes	10	15	0.6	0.17	1.8	0.3264
No	12	10	1.0	Ref.		
Use insecticide spray within the previous year (n = 47)						
Yes	11	11	1.3	0.4	4.2	0.6832
No	11	14	1.0	Ref.		
Use insecticide pour-on within the previous year (n = 47)						
Yes	9	9	1.2	0.4	4.2	0.7317
No	13	16	1.0	Ref.		
Use insect growth regulator (IGR) in mineral within the previous year (n = 47)						
Yes	10	13	0.77	0.23	2.5	0.6569
No	12	12	1.0	Ref.		
Use injectable dewormers within the previous year (n = 47)						
Yes	18	20	1.13	0.25	5.1	0.8752
No	4	5	1.0	Ref.		
Use pour-on dewormers within the previous year (n = 47)						
Yes	17	18	1.32	0.34	5.2	0.6818
No	5	7	1.0	Ref.		
Use oral dewormers within the previous year (n = 46)						
Yes	9	10	0.97	0.3	3.3	0.9587
No	13	14	1.0	Ref.		
Use CTC-medicated feed or mineral for BA prevention/control within the previous year (n = 47)						
Yes	20	13	9.2	1.7	50.7	0.0118
No	2	12	1.0	Ref.		
Use conditionally-approved BA vaccine within the previous year (n = 45)						
Yes	6	3	2.5	0.5	12.2	0.2490
No	16	20	1.0	Ref.		

Figure 1: Choropleth map of the state of Mississippi showing the distribution of participant herds (n = 47) by county.



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