PEER REVIEWED

Failure to Eliminate the *Anaplasma marginale* Carrier State in Beef Cows Following Multiple Treatments with Long-acting Injectable Oxytetracycline

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Abstract

Thirty-four multiparous beef cows naturally infected with *Anaplasma marginale* and confirmed as both seropositive and polymerase chain reaction (PCR) positive for the organism were randomized to treatment. Five cows served as untreated controls, and 29 were treated three times at three-day intervals with a long-acting oxytetracycline formulation at a dose of 10 mg/lb (22 mg/kg). On day 14, 100% of control cows and 90% of treated cows were found to have *A. marginale* present by PCR testing. All control cows and 86% of treated cows were found to have *A. marginale* present by PCR testing on day 74. PCR results suggest that utilization of a three-dose regimen of injectable long-acting oxytetracycline at three-day intervals was not effective for eliminating the carrier state of *A. marginale* from naturally infected animals.

Keywords: bovine, anaplasmosis, oxytetracycline, PCR

Résumé

Un total de 34 vaches de boucherie multipares naturellement infectées par *Anaplasma marginale*, que l'on a confirmé séropositives et positives face à cet organisme avec le test de la réaction en chaîne de la polymérase (RCP), ont été allouées aléatoirement à des traitements. Il y avait cinq vaches non-traitées témoins alors que 29 autres ont été traitées trois fois à trois jours d'intervalle avec une préparation d'oxytétracycline à longue durée d'action à la dose de 10 mg/lb (22 mg/kg). Avec l'aide du test RCP, on a détecté *A. marginale* au jour 14 chez 100% des vaches témoins et chez 90% des vaches traitées. Au jour 74, on a détecté le même organisme avec le même test chez toutes les vaches témoins et 86% des vaches traitées. Les résultats du test RCP suggèrent que l'utilisation d'un programme d'injection de trois doses d'oxytétracycline à longue durée d'action à trois jours d'intervalle n'a pas permis d'éradiquer l'état de porteur de *A. marginale* chez les animaux infectés naturellement.

Introduction

Bovine anaplasmosis occurs when red blood cells become infected with the rickettsia *Anaplasma marginale*. Once infected, cattle serve as reservoirs. The disease is spread not only by insect vectors such as ticks and biting flies, but also by fomites like multi-use needles, ear tagging equipment and tattoo pliers.[2,8] With previous cost estimates of $100 million annually and current estimated annual losses of over $300 million, anaplasmosis has a significant economic impact on producers.[5,7,9] These costs include production and death losses, treatment costs and restrictions on international trade.[6,9,10]

Treatment with long-acting injectable oxytetracycline has long been recommended as one method to clear the carrier state from infected cattle.[6,10] In older studies, *in vitro* diagnostic methods, such as the complement fixation and card agglutination tests, were used to assess success of treatment with long-acting oxytetracycline. Magonigle *et al* and Roby *et al* reported that treatment with tetracycline was an effective method to clear the carrier state.[6,10] However, newer more sensitive *in vitro* diagnostic methods, such as competitive enzyme-linked immunosorbent assay (cELISA) and polymerase chain reaction (PCR), detect the presence of deoxyribonucleic acid (DNA) found in the *A. marginale*

organisms themselves and enhance detection of antibodies to the organisms. Utilizing cELISA and PCR techniques, Coetzee *et al* demonstrated that treatment with injectable long-acting oxytetracycline was ineffective for clearing *A. marginale* infection.[2]

The objective of this study was to determine if a three-treatment, long-acting injectable oxytetracycline regimen would clear the carrier state from beef cows infected with *A. marginale* as determined by PCR diagnostic methodologies.

Materials and Methods

Animal background

A predominantly Angus, commercial beef herd with 236 multiparous cows grazing native pasture, and known to be naturally infected with *A. marginale,* was initially screened for antibodies to the organism utilizing the card agglutination test; 75 animals tested positive. Sixty-three of the 75 animals were found positive when cELISA and PCR follow-up testing was conducted 20 days prior to the start of the study (day -20). All study animals had previous access to a free-choice, medicated mineral mix containing 4.25 g chlortetracycline (CTC)/lb (9.36 g/kg) from mid-May through mid-September, prior to initial serological screening. Consumption of the mineral mix during that period was approximately 0.25 lb/hd/day (114 g/hd/day), which provided an average of 1.06 g CTC/hd/day. Access to the mineral mix was removed 241 days prior to initiation of study treatments. Precautions were not taken to avoid needle-borne transmission of *A. marginale* during late-October herd vaccinations.

Randomization

Thirty-four of the 63 mature cows confirmed anaplasmosis positive by cELISA and PCR testing were randomly selected for use in the study, using a computer-generated program. The remainder of the animals were retained for use in another study. At the start of the study (day 0), five of the 34 cows were randomly allocated to the control group, and the remaining 29 were assigned to the oxytetracycline treatment group.

Treatments

Cows in the oxytetracycline-treated group were dosed based upon an average estimated weight of 1,200 lb (544 kg). This dose regimen was selected to mimic ranch practices commonly used when producers do not have scales, but instead rely on the estimated average weight to treat all animals in the group.[2] Actual cow weights ranged from 832 to 1,337 lb (377 to 606 kg). Each cow received 5 mL /100 lb BW (10 mg/lb; 22 mg/kg BW, 60 mL total) of a long-acting, injectable oxytetracycline product containing 200 mg/mL of oxytetracycline.[a] Initial treatment was administered on day 0 and repeated at three-day intervals for a total of three treatments (Table 1). All injections were given subcutaneously in the neck utilizing a syringe and new 16 gauge x 3/4 inch (1.90 cm) disposable needle for each animal. The 60 mL dose was divided among four injection sites on each treatment day. Treatments were administered in the left side of the neck on day 0, the right side of the neck on day 3, and the left side of the neck on day 6.

Animal management

Cows were managed as a single unit on a single native grass pasture following treatment. There were no neighboring animals that could serve as sources of reinfection, and no new animals were introduced into the herd during the experiment. All study animals were treated topically for external parasites using a synthetic pyrethroid on October 5. No other parasiticides were used on study animals during the fall or winter months prior to the beginning of the experiment. Water was available *ad libitum*. Animals were observed daily by herdsmen for signs of disease or other health related issues. Evidence of disease would have resulted in animals being removed from the study, however, none of the study animals required treatment with any additional antimicrobial products following initial screening or during the study period. During springtime herd vaccinations, a new needle was used for each product administered to each animal as a precaution to prevent needle-borne transmission of *A. marginale*.

Sample collection and evaluation

Initial card test screening was completed during late December and early January. Blood samples were taken from card test-positive animals on April 22, 20 days prior to initiation of treatment (day -20; Table 1). All treated animals were given the first treatment on May 13 (day 0). Day 14 samples were taken on May 27, and day 74 blood samples were collected on July 25. Blood samples were sent to Washington State University[b] for PCR testing on days -20, 14 and 74. Blood sample collection on day 14 coincided with the approximate start date of the season when vector-borne trans-

Table 1. Schedule of events.

Day	Date	Blood collected for PCR analysis	Treatment with oxytetracycline
-20	April 22	X	
0	May 13		X
3	May 16		X
6	May 19		X
14	May 27	X	
74	July 25	X	

mission of *A. marginale* would typically begin in northeast Kansas. Day 74 blood samples were drawn during the biting fly vector season. This closely resembles the environments encountered in beef production settings in the United States.

Data management and analysis

The proportion of negative animals for each treatment group was determined separately for each time period. Standard statistical software was used to determine if the proportion of negative animals differed between groups (PEPI, Version 4.0, Sagebrush Press, Las Vegas, NV). Due to the limited number of negative animals, the Mid-P exact method was used instead of Chi-square tests.

Results and Discussion

Moderate to severe injection-site swellings were observed on the majority of animals following each treatment day, and animals were reluctant to re-enter the treatment facility on one or more of subsequent treatment days. All swellings subsided within four weeks following the last treatment.

PCR test results by day of study are shown in Table 2. On day 14, all five control animals (100%) and 26 of 29 oxytetracycline-treated animals (90%) were PCR positive for the presence of *A. marginale* organisms. There was no statistical difference between groups (P=0.69), thus no evidence that the oxytetracycline treatment regimen was effective in clearing the *A. marginale* infection by day 14. On day 74, all control animals (100%) and 25 of 29 oxytetracycline-treated animals (86%) were PCR positive for the presence of *A. marginale* organisms. Similarly, on day 74 there was no statistical evidence suggesting repeated oxytetracycline treatments were effective for clearing the *A. marginale* infection (P=0.74).

The PCR results in our study suggest that *A. marginale* was still present in some animals on both days 14 and 74 following aggressive treatment with long-acting oxytetracycline. This is contrary to results reported by Magonigle *et al* who found successful elimination of persistent infections following administration of 9 mg/lb (20 mg/kg) of oxytetracycline intramuscularly (IM) four times at three-day intervals.[6] Similarly, Roby *et al* administered two injections (9 mg/lb; 20 mg/kg) of a long-acting injectable oxytetracycline seven days apart, and concluded that this treatment was sufficient to eliminate the carrier state of *A. marginale*.[10] Swift and Thomas reported successful clearance of persistent infections using 9 mg/lb (20 mg/kg) of long-acting oxytetracycline administered IM on three occasions, three days apart, and on four occasions, three days apart.[11]

Table 2. Efficacy of treatment for *Anaplasma marginale* as indicated by study day.

	Treatment		
	Control	Long-acting oxytetracycline	
Study day	PCR+ (%)	PCR+ (%)	P-value
-20	5/5 (100)	29/29 (100)	-
14	5/5 (100)	26/29 (90)	0.69
74	5/5 (100)	25/29 (86)	0.74

Previous studies frequently used the complement fixation (CF) and card agglutination tests to determine if treatment with oxytetracycline was effective for eliminating the carrier state of *A. marginale*. However, Bradway *et al* demonstrated that the sensitivity and specificity of the CF test was 20% and 98%, respectively.[1] When this is compared to the sensitivity of 96% and specificity of 95% demonstrated for the cELISA test,[12] it suggests that previous studies may have incorrectly concluded that CF negative serologic test results indicated successful chemosterilization. Results of our study are similar to those reported by Coetzee *et al,* who found that injectable long-acting oxytetracycline did not eliminate *A. marginale* infection from artificially infected steers, as measured using PCR diagnostics.[2]

Because the insect vector season had commenced prior to day 74, and because two cows which were PCR negative on day 14 were found to be PCR positive on day 74, it is possible that reinfection of some animals may have occurred. However, our results are supported by those reported by Goff *et al*.[3] In their report, chemosterilization of 64 cattle naturally infected with a Washington strain of *A. marginale* was attempted using 9 mg/lb (20 mg/kg) of long-acting oxytetracycline administered IM on four occasions, three days apart. Animals were maintained in a tick-free environment during the time of year when there was no fly activity. One month after treatment, two animals were identified as positive using the DNA probe. Two months after treatment, samples from two different animals negative when tested one month after treatment were positive when using the DNA probe.

Data from these studies suggest that oxytetracycline does not eliminate persistent *A. marginale* infections in all cattle. Some animals may have been cleared of infection, but in the absence of subinoculation data it is not possible to definitively ascertain successful chemosterilization in carrier cattle.

Conclusion

Injection of three doses (5 mL /100 lb BW per dose, 10 mg/lb; 22 mg/kg BW) of long-acting oxytetracycline

at three-day intervals was not effective for eliminating the carrier state of *A. marginale* from naturally infected, mature beef cows when evaluated using PCR diagnostic methods.

Endnotes

[a] Liquamycin® LA-200®, Pfizer Animal Health, New York, NY 10017

[b] PCR testing provided by Guy Palmer, DVM, PhD, Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA 99164

Acknowledgements

Contribution No. 06-364-J from the Kansas Agricultural Experiment Station, Manhattan.

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Abstract

Resynchronization of estrus in beef cattle: Ovarian function, estrus and fertility following progestin treatment and treatments to synchronize ovarian follicular development and estrus

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Can Vet J (2007) 48:49-56

The objective was to optimize rebreeding of nonpregnant, previously inseminated beef cattle. In Experiment 1, 43 cows received a used intravaginal progesterone-releasing insert (IVPRI; Days 0-7) 12.3 d after ovulation and received concurrently no treatment, 100 μg gonadotropin releasing hormone (GnRH), 1 mg estradiol cypionate (ECP), or 150 mg progesterone. Emergence of a new ovarian follicular wave was most synchronous ($P<0.0001$) in the GnRH group. In Experiment 2, 675 heifers were given GnRH or no treatment on Day 0, fed melengestrol acetate (MGA; 0.5 mg/head/d) from Days 0-5 (Day 0 = 13-14 d after timed insemination; TAI), given 0.5 mg ECP or nothing on Day 7, and reinseminated 6-12 h after onset of estrus. Estrus was more synchronous ($P<0.05$) in heifers given GnRH versus no treatment on Day 0. In Experiment 3, 317 TAI heifers were resynchronized with either MGA or a used IVPRI with or without ECP on Day 7; estrus was more synchronous ($P<0.05$) and pregnancy rates were higher (54.1% versus 39.2%, $P<0.05$) in heifers given a used IVPRI than those fed MGA. For resynchronization of heifers, pregnancy rates were not significantly improved with GnRH treatment, but were higher with a used IVPRI than with MGA.