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Failure to eliminate the carrier state of Anaplasma marginale by using long-acting injectable oxytetracycline

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FAILURE TO ELIMINATE THE CARRIER STATE OF *ANAPLASMA MARGINALE* BY USING LONG-ACTING INJECTABLE OXYTETRACYCLINE

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Summary

Thirty-four *Anaplasma marginale* seropositive cows from a herd of 236 were allocated to treatments: 5 animals served as untreated controls, and 29 animals were treated with three injections of long-acting oxytetracycline at three-day intervals. Fourteen days after initiation of treatment, 100% of control cows and 89% of treated cows were found to have *Anaplasma marginale* present. Seventy-four days after initiation of treatment, 100% of control cows and 86% of treated cows were found to have *Anaplasma marginale* present. Use of injectable long-acting oxytetracycline was not effective in eliminating the carrier state of *Anaplasma marginale* from infected animals.

Introduction

Treatment with long-acting injectable oxytetracycline has long been recommended as one means of clearing the carrier state of *Anaplasma marginale* from infected cattle. Older diagnostic methods, such as the complement-fixation test and card-agglutination test have indicated the success of such treatment. Newer diagnostic methods, such as enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) have enhanced the ability to assess the presence of antibodies to those organisms or the organisms themselves. The objective of this study was to use ELISA and PCR diagnostic methodologies to determine the efficacy of three injections of long-acting injectable oxytetracycline.

Experimental Procedures

A commercial cow herd of 236 animals was screened for antibodies to *Anaplasma marginale* by using the card-agglutination test; 75 animals tested positive. Sixty-three of these 75 animals were found to be positive for *Anaplasma marginale* when confirmatory testing with both ELISA and PCR technology was conducted 20 days before the start of the study (day -20). Twenty-nine of the 63 positive animals were reserved for another study, and 34 animals were included in this study. At the start of the study (day 0), 5 of the 34 animals were randomly allocated to a control group, and the remaining 29 were allocated to the oxytetracycline treatment group. On the basis of an average estimated weight of 1200 lb, animals in the oxytetracycline-treated group received 60 mL of a long-acting, injectable oxytetracycline product containing 200 mg/mL oxytetracycline solution. All injections were given subcutaneously in the neck, with the 60 mL dose being distributed among four injection sites on one side of the neck on each treatment day. Treatment was repeated at three-day intervals for a total of three treatments per animal. Blood samples were taken from all animals 14 days after initiation of

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treatment (day 14), and again 60 days later (day 74). Blood samples were forwarded for PCR testing on day 14 and 74 and for ELISA testing on day 74.

Results and Discussion

On day 14, 5 of 5 control animals (100%) and 25 of 28 oxytetracycline-treated animals (89%) were PCR positive for the presence of *Anaplasma marginale* organisms (Table 1). The sample from one oxytetracycline-treated cow was lost from the samples submitted for PCR testing on day 14. ELISA testing was not conducted on samples collected on day 14 because this testing method measures the serological status of animals, which was not expected to change that rapidly after death of *Anaplasma marginale* organisms. On day 74, 2 of 5 control animals (40%) and 16 of 29 oxytetracycline-treated animals (55%) were ELISA positive for antibodies to *Anaplasma marginale*, whereas 5 of 5 control animals (100%) and 25 of 29 oxytetracycline-treated animals (86%) were PCR positive for the presence of *Anaplasma marginale* organisms.

All study animals had access to a free-choice mineral mix containing 4.25 g chlortetracycline/lb mix during the period of mid-May through mid-October, before initial serological screening. Consumption of the mineral mix during that period was approximately 0.25 lb cow daily. Precautions were not taken to preclude the needle-borne transmission of *Anaplasma marginale* during late-October herd vaccinations. Initial serological screening was completed during the months of December and January. Study animals were not treated with any antimicrobial product by injection or feed additive after initial screening or during the study period. Precautions were taken to prevent the likelihood that needle-borne transmission of *Anaplasma marginale* would occur during springtime vaccinations. Day 14 blood samples were drawn before the start of the biting fly season, when *Anaplasma marginale* transmission could occur. Day 74 blood samples were drawn after the biting fly season was under way.

Repeated injections of long-acting oxytetracycline were not effective in eliminating the carrier state of *Anaplasma marginale* from infected animals (Table 1). PCR testing was able to detect the presence of *Anaplasma marginale* organisms even when ELISA testing did not detect serological evidence of their presence (Table 1).

The declining ELISA results from day -20 to day 74 in both control and oxytetracycline-treated animals (Table 1) were unexpected and suggested a degree of non-treatment-related recovery or reversion to non-seropositive status in both groups. However, the PCR results indicated that *Anaplasma marginale* organisms were present on both day 14 and day 74. If treatment with three injections of long-acting oxytetracyline had been successful in eliminating the carrier state of *Anaplasma marginale*, PCR test results were expected to have been negative on both test dates.

Table 1. Lack of Efficacy of Treatment with Long-Acting Oxytetracycline as Indicated by Number and Percentage of Animals Still Positive for *Anaplasma marginale* on the Basis of ELISA and PCR Testing on Each Study Day

	Control		Long-Acting Oxytetracycline	
	ELISA	PCR	ELISA	PCR
Study Day	Positive (%)	Positive (%)	Positive (%)	Positive (%)
−20	5/5 (100)	5/5 (100)	29/29 (100)	29/29 (100)
14		5/5 (100)		25/28 (89)
74	2/5 (40)	5/5 (100)	16/29 (55)	25/29 (86)