Blood Levels of Oxytetracycline After the Use of a Long-Acting Formulation to Eliminate the Carrier State of Bovine Anaplasmosis

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Introduction

Research workers have long endeavored to find practical chemotherapeutic regimens for eliminating the carrier state of bovine anaplasmosis. In 1975 it was reported that the intravenous injection of oxytetracycline at the rate of 22 mg/kg/day for five days could eliminate the carrier state of anaplasmosis in infected cattle (1). While this regimen is more practical than 10-12 daily injections, previously recommended as effective, it would be less expensive and more desirable to have preparations of anaplasmacidal drugs which would require even less frequent handling of cattle to achieve elimination of the carrier state. As long as carriers of *Anaplasma marginale* occur there is the potential for outbreaks of clinical anaplasmosis with its attendant morbidity and mortality. The insect and tick vectors of this disease are, at best, poorly controlled by insecticides and acaracides. Vaccination with killed products may reduce the economic losses, but vaccination does not yet afford a means to prevent the spread of infection and the further development of *A. marginale* carriers.

The purpose of this paper is to present data on the blood plasma levels of oxytetracycline which resulted when cattle were injected intramuscularly (IM) once a week with a strengthened and improved formulation of oxytetracycline containing 200 mg/ml of base drug. One injection of this formulation has been reported to have an inhibitory effect on the parasitemia in acute anaplasmosis equivalent to three daily injections of oxytetracycline containing 50 mg/ml (2). Also, the former formulation has been recently reported to effect the elimination of the carrier state of bovine anaplasmosis when administered IM at seven-day intervals (3).

Materials and Methods

Twelve intact Holstein-Friesian yearling cattle weighing from 254-343 kg were treated with Liquamycin/LA-200. All had been experimentally infected with *A. marginale* two months previously, allowed to recover spontaneously from acute anaplasmosis, and had become inapparent carriers of the parasite. Card agglutination tests were positive, and complement-fixation titers averaged 1:320 at the time therapy was started. Four cattle were treated twice at seven-day intervals by the IM injection of 20 mg/kg of the oxytetracycline in the mid-cervical region; four were treated three times, and four were treated four times in a like manner. Prior to injection of the drug, a blood sample (heparinized) was collected for plasma and subsequent oxytetracycline assay. These samples were then centrifuged, the plasma removed and frozen at -10°C. Bioassays* for oxytetracycline were conducted after the end of the experiment by the standard method. Two additional infected cattle were treated by IV injections with 11 mg/kg daily for 12 days of oxytetracycline as contained in Liquamycin (50 mg/ml). This was done to establish that the strain of *A. marginale* used was sensitive to oxytetracycline. Two other infected cattle were unmedicated and served as controls on the latency of the carrier state of the parasite. After therapy, blood samples for serology and hematocrit determinations were collected every two weeks. Eighty-three days after therapy 80 ml of whole blood from each animal was inoculated IV into a splenectomized susceptible test calf to test for the presence of *A. marginale* in the principals. The test calves were bled weekly. Their blood was examined for the presence of anaplasma bodies using a Giemsa stained slide, and hematocrit determinations were done.

Results

Prior to this study it had been found that sustained blood levels of oxytetracycline (OTC) (>0.2 mcg/ml) were observed over a three- to five-day period following a single intramuscular injection of Liquamycin/LA-200 at 20 mg/kg. In a composite study of 200 cattle, detectable blood levels were present in some cattle as long as 144 hours post-injection,

*Microbiological Agar Diffusion Assay for Oxytetracycline in Animal Blood Serum. Pfizer, Inc., Standard Test Procedure No. 012.15. Vigo Quality Control, Terre Haute, Ind.

while other cattle did not have detectable levels at 96 hours post-injection. The average blood depletion level of OTC following the intramuscular injection at 20 mg/kg in divided dosage sites in 200 cattle is shown in Table 1.

In this study bioassays of plasma from 12 yearlings treated either 2, 3, or 4 times with Liquamycin/LA-200 are presented in Table 2. Detectable OTC plasma levels were found in 10 of 12 samples tested 7 days after one previous treatment; 6 of 8 samples 7 days after two previous treatments; 4 of 4 samples 7 days after three previous treatments; and 4 of 4 samples 7 days after four previous treatments.

Bioassays were conducted daily for 11 days, beginning one day after the first treatment in the two calves receiving 11 mg/kg Liquamycin (50 mg/ml) for 12 days. These results are shown in Figure 1. There were no noticeable trends up or down in the plasma levels for this period of time. The average level was 1.76 mcg/ml (±0.20).

The blood (80 ml) from each of the 12 cattle was found non-infectious for *A. marginale* when inoculated into susceptible splenectomized test calves 83 days after treatment with Liquamycin/LA-200 (3). The two control calves receiving 12 daily injections of 11 mg/kg Liquamycin (50 mg/ml) IV were also shown to be free of infection 83 days after treatment. The two carrier, non-treated calves retained their infection during this time.

Table 1

Mcg/ml of Oxytetracycline - Hours Post-Injection						
4	24	48	72	96	120	144
3.4	2.3	1.1	0.5	0.3 (90%)*	0.2 (63%)*	0.1 (48%)*

*Percent of cattle with detectable blood levels.

Table 2

Oxytetracycline Plasma Levels (mcg/ml) 7 Days After 1, 2, 3, and 4 Intramuscular Injections of Liquamycin/LA-200 at the Rate of 20 mg/kg.

7 days after 1 injection	7 days after 2 previous injections	7 days after 3 previous injections	7 days after 4 previous injections
0.20 mcg/ml ±0.029 mcg/ml	0.24 mcg/ml ±0.025 mcg/ml	0.25 mcg/ml ±0.041 mcg/ml	0.30 mcg/ml ±0.06 mcg/ml
Nos. = 10/12	6/8	4/4	4/4

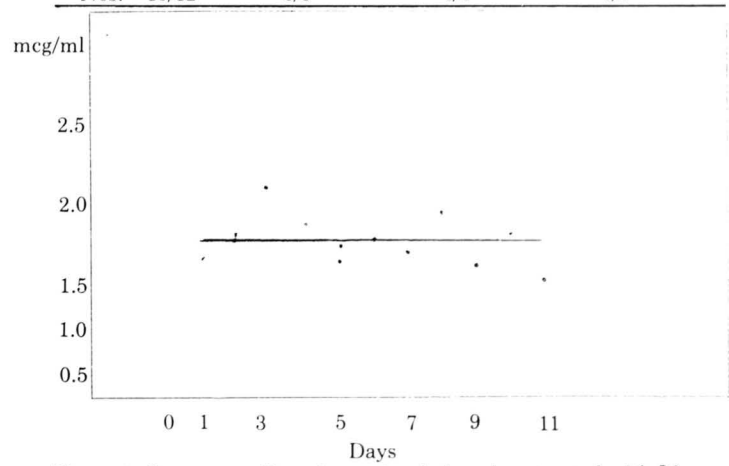

Figure 1. Oxytetracycline plasma levels in calves treated with Liquamycin (50 mg/ml).

Discussion

The use of tetracycline drugs at relatively high dosage levels for varying periods of time has been an accepted procedure for eliminating A. marginale **infection. Either prolonged feeding of chlortetracycline or daily injection of oxytetracycline have been used for this purpose in a number of earlier studies. The present study suggests that the use of a long-acting oxytetracycline will effectively eliminate the carrier state of sensitive strains of** A. marginale**. Also, the present study shows that in a limited number of cattle, detectable blood levels were present in 10 of 12 cattle seven days after one treatment with the long-acting formulation of oxytetracycline. It will be necessary to further evaluate the use of this treatment regimen to determine if similar results can be achieved under field conditions.**

References

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