Effects of a Sustained-Release Oxytetracycline Bolus for Anaplasmosis Carriers

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Summary

Sustained-release oxytetracycline boluses were administered at dosages ranging from 1.0-3.0 mg active ingredient (AI)/kg body weight to naturally and experimentally infected bovine *Anaplasma* carriers. Bolus efficacy was evulated by serology and hematologic parameters and by subinoculation of blood from cows into susceptible, splenectomized calves. Sustained-release boluses were effective in reducing complement-fixation (CF) titers at all drug levels. One group of naturally infected cows that were carriers converted to CF negative. However, subsequent subinoculation of blood from these cows into susceptible calves caused anaplasmosis and demonstrated that the organism had not been eliminated from the animals.

Anaplasmosis is an acute, sub-acute or chronic disease of cattle caused by the intraerthrocytic parasite, *Anaplasma marginale* Theiler. Cattle recovering from the acute infection are generally resistant to further clinical episodes of the disease because very low levels of the organism persist in a carrier state which confers immunity. The carrier state is clinically inapparent but is important because carrier cattle are primary reservoirs of *A. marginale* and have been shown experimentally to serve as an infective source for ticks.(1) Regardless of vaccination history, carriers are a source of contamination to unprotected herds where potential vectors are active. Elimination of the carrier state of anaplasmosis is therefore an important preventative measure against herd infection and enzootic spread of the disease.

Tetracyclines have been tested extensively against the carrier state(2-5) and have been found to be successful in eliminating the infectious agent. Chlortetracycline administered orally at 0.23 mg/kg (0.5 mg/lb) for 120 consecutive days proved effective in eliminating infections.(6) More consistent removal of carrier infections was obtained with higher levels of drug. Franklin, at al(7) found that the organism could be eliminated when chlortetracycline was administered at 2.3 mg/kg (5 mg/lb) for 60 days.(4) Magonigle, et al(8) reported that IV injections of oxytetracycline at 22 mg/kg for 5 consecutive days would eliminate the infectivity and results of Roby, et al(9) demonstrated that 2 to 4 IM injections of a long-acting oxytetracycline formulation (L-200) 7 days apart eliminated the infection from recently infected yearling cattle. Effective and practical implementation of such treatment regimes has been limited due to variability associated with intake of tetracyclines when fed and generally injections of large and multiple dosages are required.

Recently, a sustained-release oxytetracycline bolus(10) was developed in our laboratory and has been shown to prevent clinical anaplasmosis and disease transmission by adult, infected ticks.(11) The bolus is retained within the rumeno-reticular sac of the bovine and sustained-release of oxytetracycline occurs as the bolus erodes due to normal rumination. This method of treatment has demonstrated several advantages over conventional methods. The bolus provides regular erosion with a reasonably consistent release of drug, thereby maintaining therapeutic blood levels for 60 days. Also, this treatment regime is more compatible with most management programs because it results in less animal excitation and does not cause tissue irritation encountered with IV and IM injections.

The purpose of this study was to determine the efficacy of the sustained-release oxytetracycline bolus in the

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elimination of the carrier state of *A. marginale* in both experimentally and naturally infected cattle.

Methods and Materials

Experimentally infected cattle—Seven 6-9 year old Holstein, Ayrshire and Jersey dairy cows sero-negative for anaplasmosis by the complement-fixation (CF)[a] test were stanchioned and exposed to *A. marginale* by allowing 75 pairs of infected *Dermacentor andersoni* Stiles to feed on each animal. After allowing ticks to complete feeding, cattle were maintained under drylot conditions and fed a cottonseed hull based ration(12) to minimize gain during the test period. Cattle were monitored for patent infection by examination of Wright's-stained blood slides, determination of the packed cell volume (PCV) and CF serology. After detection of marginal bodies in the peripheral blood, cattle were monitored daily. In order to prevent death loss of cattle once infected, animals were given oxytetracycline IM at 0.25-0.45 mg/kg of body weight until the parasitemia values began to decline.

Following transition from acute anaplasmosis to the chronic stage, carrier cattle were weighed and randomly divided into 4 groups. Groups I, II and III were comprised of 2 animals/group and received oxytetracycline at a dosage of 1.0, 1.5 and 2.0 mg active ingredient/kg body weight, respectively, via a 20% oxytetracycline bolus. One animal, comprising Group IV was not bolused and served as a control. Blood samples were collected from each cow on a weekly basis, evaluated by CF serology and blood from CF negative cattle was sub-inoculated into susceptible splenectomized calves.

Naturally infected cattle—Fifteen Hereford and Angus cattle, determined carriers of anaplasmosis by a repeated positive reaction to the CF test, were obtained from an anaplamosis enzootic area in south Texas and maintained on 160 acres of native pastureland in Payne Co., Ok. Animals were weighed 3 times prior to bolus administration to determine proper drug treatment level and then randomly divided into 5 groups with 3 animals/group. Groups I, II, III and IV received oxytetracycline at theoretical dosage rates of 1.5, 2.0, 2.5 and 3.0 mg/kg of body weight, respectively. Since the 20% oxytetracycline bolus had a longevity of ca 60 days, (10) additional bolusing was administered at the end of this period to provide a 120 day treatment. Group V cattle were not bolused and served as untreated controls.

Blood samples were collected on a weekly basis for evaluation by CF serology. Following the 120 day treatment and 60 additional days to allow clearance of any drug residues which may have occurred, 2 animals selected at random from each group determined either suspicious or negative for anaplasmosis were bled and sub-inoculation procedures were conducted with susceptible, splenectomized calves. A total of 175 ml of blood pooled from each treatment group was treated with neoarsphenamine as described by Jones, et al(13) and injected IV into calves. The calves were then monitored for patent infection by Wright's stained blood slides, determination of the PCV, and CF serology.

Results

Experimentally infected cattle—Cattle exhibited acute infections which were characterized by a normal prepatent period of 28 days and a clinical disease with a mean (X) maximum parasitemia of 21.3%. During latent infection, CF titers ranged from 40-160R with parasitemias of 1-4%. Table 1 summarized the results of treatments using the 20% oxytetracycline bolus. Sixty days post-administration of oxytetracycline boluses, the CF titers remained positive (4+) in all groups. Following the next 60 day period, Groups II and III began to convert to a suspicious CF (2+) titer. Sub-inoculation procedures conducted 120 days post-treatment with Groups II and III demonstrated that elimination of the causal organism did not occur at these dosage levels. The splenectomized calves had a mean (X) prepatent period of 29 days and converted to a positive (4+) CF titer at an average of 3 weeks post-inculation.

Table 1. Response of experimentally infected cattle to sustained-release oxytetracycline bolus therapy for elimination of anaplasmosis carrier infections, Payne Co., Oklahoma, Summer, 1980.

Group	Treatment (mg/kg)	Mean ($\bar{X}$) CF titers			
		Pre-treatment	Days Post-treatment		
			60	90	120
I	1.0	80R	40R	10R	5R
II	1.5	40R	20R	5R	< 5S
III	2.0	160R	80R	< 5S	< 5S
IV	Control	80R	40R	40R	20R

Naturally infected cattle—Drug therapy design and influence of bolus treatment on the carrier state of those animals maintained under field conditions are summarized in Table 2. These animals were exposed naturally to *A. marginale* as yearlings and had an existing latent infection with CF titers ranging from 10-20R and coinciding parasitemias of <1%. Group I showed a suspicious (3+) CF titer within the first 30 days post-treatment. Thereafter, this

[a]*CF test performed by Oklahoma Disease Diagnostic Lab, Stillwater, Oklahoma.*

group was found to be either suspicious or negative for anaplasmosis throughout the treatment period and remained so during the additional 60 day post-treatment period. Group II exhibited positive (4+) CF titers for 120 days at which time 2 animals had a suspicious (2+) CF titer. Animals in Groups III and IV remained positive (4+) for the disease during the 120 day treatment period, however between days 120 and 180 these animals occasionally converted to a suspicious CF (3+) titer. Results from sub-inoculation procedures utilizing splenectomized calves with Groups I and II, demonstrated a positive (4+) CF titer from Group I while Group II did not convert to a positive CF titer. However, *Anaplasma* bodies (1-3%) were detected by the Wright's-stained blood slides following a 63-day prepatent period.

Table 2. Design and influence of sustained-release oxytetracycline bolus treatment on naturally infected *Anaplasma* carrier cattle, Payne Co., Oklahoma, Summer, 1980.

Group	Treatment (mg/kg)	Mean ($\bar{X}$) CF titers			
		Pre-treatment	Days Post-treatment		
			60	120	180
I	1.5	15R	< 5S	< 5S	Negative
II	2.0	10R	10R	< 5S	< 5S
III	2.5	20R	10R	20R	10R
IV	3.0	15R	10R	5R	10R
V	Control	10R	20R	10R	10R

Discussion

The treatment of experimentally infected cattle with dosage levels of 1.0 - 2.0 mg/kg reduced CF titers in all treatment groups but did not eliminate the disease agent. This was most likely due to insufficient blood levels of oxytetracycline. During concurrent studies,(14) oxytetracycline boluses with densities less than 1.8 g/cm^3 were found to move between the reticulum and ventral sac of the rumen. This movement resulted in release rate deviation from delivery expected when boluses remain in the reticulum. Consequently, oxytetracycline blood levels were probably lower because boluses used in this study probably migrated within the rumen.

Bolus therapy to naturally infected cattle at dosages of 1.5 to 3.0 mg/kg similarly reduced positive CF titers. Several animals converted to CF negative. We anticipated that CF titers would decline as the drug level administered increased. This did not occur in these studies and was probably due to several unanticipated factors. The oxytetracycline bolus developed for animal health purposes(10) was intended to be administered to cattle at a level of 2-4 boluses per animal. In order to deliver oxytetracycline at 2.5 to 3.0 mg/kg of body weight, 7-8 boluses were required to treat animals weighing over 500 kg. As the number of boluses increased, the closeness of individual boluses probably caused release rates(14) to exceed the expected delivery. This consequently caused a decrease in bolus longevity and also, decreased the length of the treatment period. In addition to the recently noted phenomenon mentioned above, the type of diet was also found to influence bolus erosion.(14) High density grain diets have been shown to facilitate bolus erosion, whereas diets of grazing cattle containing sparse particulate matter caused lower bolus erosion rates.

The sustained-release bolus system may offer an approach to anaplasmosis control which would be plagued by fewer problems than conventional control methods. For example, it should impose fewer management and labor constraints and provide an economic advantage for the producer. In addition, the predictable and consistent release of tetracycline should offer protection for up to 60 days with a single bolus application.

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