Effects of the Anticoagulant Diphenadione on Suckling Calves

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Introduction

Vampire bat parasitism on cattle has long been a source of economic loss and hardship for cattlemen of Latin America. The transmission of paralytic rabies and possibly other diseases, blood loss, myiasis, reduced production and other factors all contribute to a multimillion dollar problem.

Researchers at the Denver Wildlife Research Center (DWRC) published initial results of a systemic method for reducing vampire bat parasitism on cattle in 1972 (THOMPSON et al. 1972). This technique involves the injection of the anticoagulant diphenadione (2-diphenylactyl-1,3,indandione) into the rumen compartment of cattle. Diphenadione is absorbed into the bloodstream of the host and later ingested in lethal quantities by the vampires as they feed on blood from the cattle. Death results from internal hemorrhaging. The procedure is currently being used on a large scale in various Latin American countries for control of vampire bats. Nearly 200,000 cattle have been treated in Nicaragua since 1974 and similar programs are starting or anticipated in Colombia, Ecuador, Panama, Brazil and other countries (MITCHELL et al. 1975).

Laboratory and field observations show that 1 mg/kg doses of diphenadione present no hazard to adult cattle (THOMPSON et al. 1972). We have, however, been informed by Nicaraguan officials that 9 of 14 calves died after treatment with diphenadione (1 mg/kg). During an earlier experiment at DWRC laboratories, one of three calves dosed with 5 mg/kg of diphenadione died 5 days posttreatment.

Since diphenadione is an anticoagulant with potent antiprothrombin activity, it is potentially dangerous to all classes of mammals. Depression of the prothrombin level in the blood may occur under conditions such as Vitamin K deficiency or as a consequence of the action of anticoagulant drugs, which act as competitive antagonists of Vitamin K (COHN and MANDEL 1965). Vitamin K is known to be present in the photosynthetic portion of plants and is synthesized by bacterial action in the mammalian intestinal tract (COHN and MANDEL 1965). Mature cattle with a fully functional rumen and on a diet of plant materials probably obtain sufficient amounts of Vitamin K to preclude any adverse effects from the diphenadione treatment. On the other hand, young calves, lacking a fully

functional rumen and on a diet of milk, may not receive sufficient amounts of Vitamin K to counteract the effects of the drug. If such is the case, supplemental doses of Vitamin K should reduce the toxic effect of diphenadione in treated calves.

The objectives of this experiment were threefold: (1) to obtain further information on the effects of diphenadione on young calves; (2) to determine if injections of Vitamin K_3 would be of value if calves did exhibit higher susceptibility; and (3) to determine what effect the Vitamin K_3 would have on the toxicity to vampire bats of blood from the treated animals.

Methods and Procedures

A total of 20 animals (10 adult lactating cows and 10 suckling calves) were held in DWRC pen facilities at the Denver Federal Center. Vampire bats imported from Nicaragua were held in cages at DWRC laboratories.

The cattle were identified by number on neck chains and divided into three groups: (1) adult group (n=10), (2) calf treatment (Vitamin K) group (n=5), and (3) calf control group (n=5). The two calf groups were matched as closely as possible on the basis of weight. Weights ranged from about 50 to 149 kg and ages from about 2 to 4 months.

Each animal was restrained in a squeeze chute and the body weight estimated with a cattle weighing tape. Then each received an injection of 2 ml/100 kg of Motomco Suspension Vampiracida Difenadiona* (equivalent to 1 mg/kg of diphenadione) in the rumen. In addition, immediately after the administration of diphenadione, the five calves in the treatment group received a single intra-muscular injection (1 mg/kg) of Vitamin K_3 (menadione sodium bisulfite; Haver-Lockhart Laboratories), and the five calves of the control group received an intramuscular injection of physio-logical saline at the same dosage level.

Blood samples (about 10 ml, most of which was used for related studies) were collected from each animal immediately before treat-ment and at 24-hour intervals up to 144 hours posttreatment.

Clotting times were measured by the Quick method of prothrombin time determination (MONKHOUSE 1961).

At the 24-hour collection, about 40 ml of blood were drawn from each calf. After it was defibrinated, 30 ml were offered to each of 10 captive vampire bats for a single 24-hour feeding period. Five bats received blood from the calves treated with Vitamin K_3 and five received blood from the control calves that had received

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* Reference to trade names does not imply endorsement of commer-cial products by the Federal Government or any of its agencies.

saline. The bats had been held in captivity for 5 weeks before the experiment and had adapted to feeding on defibrinated cattle blood from glass feeding tubes suspended on their cages.

Statistical Analysis

We computed a two factor analysis of variance (3 cattle groups x 7 sampling periods, treating sampling periods as a repeated measures factor, to assess differences in prothrombin times among treatments, and then compared treatment means by using Duncan's Multiple Range Test for Unequal Numbers (WINER 1971). Before the analysis of variance was computed, three missing prothrombin values (resulting from the deaths of two animals) were estimated by the method of least squares.

We used the Fisher Exact Probability Test (SIEGEL 1956) to analyze the toxicity to vampire bats of blood from the two calf groups.

Results

Analysis of variance revealed significant ($P < .05$) differences in prothrombin clotting times for the three possible effects: Cattle groups (F=6.52; df=2/170), sampling periods (F=8.28; df=6/101), and the interaction between cattle groups and sampling periods (F=3.66; df=12/101).

Comparison of the overall means for the three cattle groups (Table 1) showed that the clotting time of the control calf group (82.3 sec) was significantly higher than that of the adult group (20.1 sec), but that neither the mean clotting times of the control calves nor the adults differed from that of the treatment calf group (46.8 sec). This pattern of means confirms that: (1) diphenadione caused a threefold increase in clotting times for calves relative to adults and (2) administration of Vitamin K_3 to diphenadione treated calves caused a general reduction in the magnitude of this effect.

Separation of the mean clotting times for all animals at the seven sampling periods showed that mean times at 72 hours (63.5 sec) and 96 hours (62.9 sec) were significantly greater than those at 0 hour (14.3 sec) and 24 hours (Table 1). Additionally, the 120-hour mean (50.3 sec) was significantly greater than the 0 hour value. This pattern of mean differences indicates that diphenadione generally lengthened clotting time for all animals and that this effect was greatest 72-96 hours after treatment.

Each group of cattle displayed a unique pattern of prothrombin response during the course of the study (Figure 1). The adults showed little change in clotting times at the seven sampling periods (i.e., clotting times gradually increased from 14.1 to 30.5 sec during the initial four periods and then began to decrease toward the baseline). Clotting times of the calves treated with Vitamin K_3 increased sharply up to the 72-hour sampling period, and

TABLE 1

Mean prothrombin clotting times in seconds (± SD) for cows and calves, at different intervals after treatment with diphenadione.

| Animal age and treatment group	Hours after treatment							Group average
	0	24	48	72	96	120	144	
Adults								
Diphenadione	14.1 (0.8)	16.6 (1.3)	24.4 (3.1)	30.1 (7.1)	21.1 (4.0)	18.1 (2.6)	15.8 (1.7)	20.1 (5.8)
Calves								
Diphenadione + Vitamin K_3	14.8 (1.0)	21.7 (4.0)	55.4 (29.7)	90.9 (43.2)	77.8 (68.9)	41.0 (19.2)	20.5 (6.0)	46.8 (41.8)
Diphenadione + saline	14.3 (0.5)	21.8 (4.4)	60.8 (21.0)	102.0 (37.2)	132.0 (65.0)	124.2 (115.0)	122.0 (180.4)	82.3 (49.8)
Average, all animals	14.3 (0.8)	19.2 (3.9)	41.3 (24.1)	63.5 (43.2)	62.9 (64.1)	50.3 (69.8)	44.7 (97.4)	

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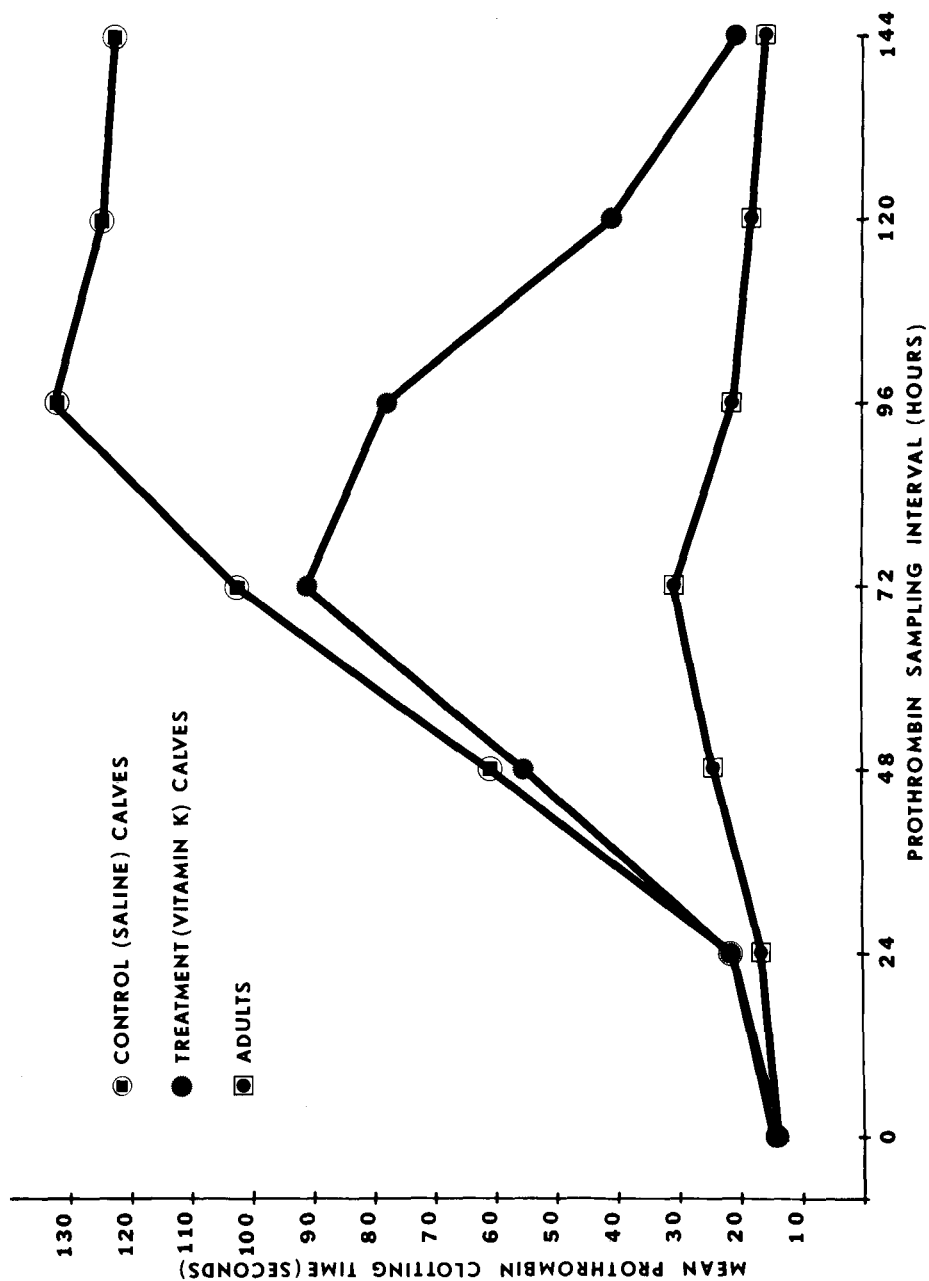
Figure 1. Mean prothrombin clotting times for test animals by treatment group.

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then decreased abruptly (i.e., clotting times increased from 14.8 to 90.0 sec in the first three periods, and fell to 46.8 sec at 144 hours). In the control calves, on the other hand, clotting times increased rapidly (from 14.3 to 132 sec) throughout the first 96 hours and decreased little thereafter, remaining above 120 sec at the 120 and 144 hour samplings.

Duncan Range Tests for these cattle groups by sampling period means revealed significant differences between the clotting times of both calf groups as compared with those of adults at 72 and 96 hours. Beyond 96 hours, however, the values for Vitamin K calves were not different from the values for adults whereas those for the control calves continued to differ significantly. Thus the administration of Vitamin K_3 foreshortened the effects of the anticoagulant.

Statistical analysis of the 24-hour blood toxicity trial with vampire bats revealed no significant difference in lethality of the blood from Vitamin K_3 calves (three of five bats died) and control calves (two of five bats died).

Discussion

Two calves, one from the Vitamin K_3 group and one from the control group, succumbed to anticoagulant poisoning. These were the youngest and smallest of the calves; each weighed less than 92 kgs. Both exhibited clinical symptoms typically associated with anticoagulant poisoning, such as stiffness or lameness in the limbs, epistaxis, and hematoma. Postmortem examinations revealed bloody pleural effusion, ascites, and extensive subcutaneous and retroperitoneal hemorrhage. Petechial hemorrhages were observed on various organs such as the lungs, liver, intestine, and thymus. The trachea and bronchi contained a pink frothy liquid.

The accepted safe limit in prothrombin time increase for humans undergoing anticoagulant therapy is two times the control value (DUFF et al. 1955). If the pretreatment prothrombin times for each group (Table 1) are accepted as the control values for the animals in the present study, both calf groups far exceeded the safe limit but the adults did not. The present results, plus the previously mentioned deaths of nine young calves in Nicaragua and the one at DWRC, offer rather convincing evidence that young calves are highly susceptible to diphenadione and that treatment of animals less than 4 months involves a high degree of risk.

One might reasonably ask why only two of the animals died, when the prothrombin times of all the calves exceeded the safe limit of twice the control value. Clearly, high prothrombin times may be, but are not always, fatal. Probably hemorrhaging occurred in all animals; however, the degree and site of hemorrhage is likely to vary among individuals, and the ultimate results (survival or death) would be highly dependent on the degree and location of the hemorrhage. Certainly a cerebral hemorrhage would have a far

more profound effect than a subcutaneous hemorrhage in a limb. Our data merely indicates a high degree of risk for young calves.

The single intramuscular injection of Vitamin K_3 as given in this study did not provide sufficient protection against diphenadione poisoning. However, further study and experimentation might not yield the same results. The time for recovery to normal or near-normal prothrombin times in the Vitamin K_3 treated calves was markedly shortened. Adjustments in time of administration, dosage level, and number and timing of doses could yield prothrombin clotting times for calves closely similar to those for adults. Field experience indicated, however, that any treatment in addition to the bat control treatment should, ideally, be applied at the same time the animals are treated for vampire control, on the basis of the practical consideration of reducing labor and handling. Therefore we limited the Vitamin K_3 treatment to a single injection given immediately after treatment with diphenadione. The severity of vampire bat parasitism, and whether or not rabies is an immediate factor, could alter these considerations.

Evidence accumulated to date indicates that (1) young calves, lacking a fully functional rumen and primarily dependent on milk for sustenance, are susceptible to diphenadione poisoning; (2) single intramuscular injections of Vitamin K_3 given at the time of treatment with diphenadione foreshortened prothrombin clotting time recovery periods but did not offer complete protection against diphenadione poisoning in young calves; and (3) blood from calves treated with diphenadione and Vitamin K_3 is as toxic to vampire bats as that from animals treated only with diphenadione. On the basis of these conclusions, we recommend that, until a satisfactory antidotal procedure is developed, the diphenadione rumen injection technique of vampire bat control not be used on cattle less than 4 months old.

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