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EFFICACY AND SAFETY OF THE SYSTEMIC METHOD OF VAMPIRE BAT CONTROL

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AND R. DANIEL THOMPSON

SUMMARY *In the systemic method of controlling vampire bats* (Desmodus rotundus), *cattle are given a single intraruminal injection of 1 mg/kg of diphenadione[4] (2-(diphenylacetyl)-1,3-indandione). This anticoagulant is rapidly absorbed into the bloodstream, and any bat that feeds from a treated animal within 72 hours receives a lethal dose. The injection is harmless to adult cattle, who receive ample vitamin K (the antidote) from their rumen bacteria and from green plants. In contrast, vampire bats are highly susceptible; the acute oral LD_{50} is 0.91 mg/kg for inactive bats and perhaps one-third that level for exercising bats. The systemic method has proved safe and highly effective in field tests in Mexico, Ecuador, and Nicaragua, and has been widely used in a National Campaign in Nicaragua, where vampire bat attacks have been reduced by more than 90 percent and bat-transmitted rabies appears to have been eliminated.*

Additional studies have shown that diphenadione residues in meat and milk from treated cattle pose no hazard to human health. Detectable residues were not found in the milk of cows dosed at the recommended 1 mg/kg level but 0.021 ppm or less was present in cows dosed at 2.75 mg/kg. Diphenadione was detected only in liver and kidney samples (0.15 ppm or less) 30 days after cattle were given 1 mg/kg doses. Rats fed the liver of treated cattle for 2-4 weeks showed no ill effects. These residue levels are minute in comparison with the minimum daily dosage of diphenadione used in human therapy.

There has been a long-standing need for elimination of the agricultural and economic losses to the people of Latin America caused by vampire bats *(Desmodus rotundus)*. These bats are carriers of paralytic rabies which is estimated to claim 2 million head of livestock annually (Constantine, 1970). In 1968, the Mexican Agriculture and Livestock Ministry's National Institute of Livestock Research and the U. S. Fish and Wildlife Service under a sponsoring agreement with the U. S. Agency for International Development cooperated in a program to find a solution to this problem. Two vampire bat control methods were developed, one a topical application of an anticoagulant to the bat (Linhart *et al.*, 1972) and the other a systemic treatment of the bat's host (Thompson *et al.*, 1972). In the systemic method bats are killed by consuming diphenadione (2-(diphenylacetyl)-1,3-indandione) circulating in the blood of treated cattle. Since 1972, personnel of the Denver Wildlife Research Center and cooperators in various Latin American countries are engaged in research and extension efforts to develop and apply these methods. Considerable emphasis has been directed at evaluating the effectiveness and safety of the systemic mehod. This paper summarizes laboratory and field data compiled in these investigations.

The Systemic Control Method

The systemic method capitalizes on the daily requirements of vampire bats for blood, the reversible binding of indandione anticoagulants like diphenadione to the blood plasma albumin, and the much greater sensitivity of vampire bats than of ruminant livestock to these drugs. A 1 mg/kg dose of diphenadione is injected into the rumen compartment of cattle and is rapidly absorbed into the bloodstream. Any vampire that feeds from a treated animal within 72 hours receives a lethal dose.

There is ample evidence that this treatment is safe for adult ruminants. Thompson *et al.* (1972) reported that a single oral dose of up to 5 mg/kg in cattle produced no observable signs of intoxication beyond a moderate increase in plasma prothrombin clotting time; these data on clotting times are summarized in Table 1. We have dosed adult cattle with up to 10 mg/kg since that time and have made the same observation. The values of Table 1 are even higher than those reported in Table 3 for adult lactating cows. Factors such as mode of administration or physiological state of the animal could be involved. Probably the most important is that the former study was conducted in January and the latter in June. Green grass was available in June whereas in January the cattle depended on alfalfa hay for their source of vitamin K.

To our knowledge, there have been no reported ill effects in any of the adult cattle treated in any of the Latin American countries. This safety for ruminants is primarily attributed to the fact that large amounts of vitamin K, the antidote for diphenadione, are synthesized by their rumen bacteria (Barnett and Reid, 1961) as well as occurring widely in the green leafy plants they consume (Harper, 1971).

In contrast, vampire bats are highly susceptible to diphenadione. Since their blood food is low in vitamin K and the only other source of this antidote is through synthesis by intestinal microorganisms, the bats can apparently "buffer" little of the anticoagulant. In a laboratory study with caged, inactive vampire bats, the single oral LD_{50} (dose statistically estimated as lethal to 50 percent of the population) was 0.91 mg/kg (Thompson *et al.*, 1972). However, our observations of treated bats indicate that body movements during flight increase the incidence of blood capillary ruptures, especially in the wings, so that active bats die from internal hemorrhaging at even lower dosages.

Recognizing the importance of exercise in vampire bats' sus-

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ceptibility to diphenadione allowed us to set a dosage in cattle as low as 1 mg/kg. At first, laboratory data indicated this dosage would be too low. The mean concentration of diphenadione in blood plasma of cattle given 1 mg/kg was 0.61, 1.0, and 0.81 ppm respectively at 12, 24, and 48 hours post-treatment. The cattle's mean hematocrit of 35.3 percent means that a 30 gram vampire bat drinking 20 ml of blood would receive 28.7, 47.2 and 38.5 percent respectively of the 0.91 mg/kg LD_{50} for inactive bats. However, in a pilot study we found indications that the lethal dosage could be reduced to about one-third that level by simply exercising the bats for 3 minutes daily. In one field study Wimsatt (1969) observed that the mean foraging time for a colony of bats was 2 hours. The probability of long periods of exercise makes the 1 mg/kg dosage, which is marginal in the laboratory, ideal in the field. Operational successes with the systemic method attest that this is the case.

TABLE 1

PLASMA PROTHROMBIN CLOTTING TIME OF ADULT CATTLE GIVEN SINGLE ORAL DOSES OF 0.5-5 mg/kg OF DIPHENADIONE

Hours posttreatment	Prothrombin clotting times in seconds (mean ± SD)			
	0.5 mg/kg n=4	1 mg/kg n=3	2.75 mg/kg n=4	5 mg/kg n=4
0	18.9 ± 2.8	17.5 ± 0.9	22.2 ± 3.2	19.8 ± 2.1
24	26.5 ± 7.2	23.9 ± 1.4	27.0 ± 3.3	30.1 ± 8.1
48	45.6 ± 19.7	52.0 ± 12.5	58.1 ± 9.5	63.3 ± 18.3
72	43.8 ± 23.6	68.3 ± 18.0	95.0 ± 9.7	124.1 ± 66.2
96	28.6 ± 10.3	40.7 ± 22.1	121.9 ± 47.9	183.6 ± 94.3
120	22.7 ± 5.2	21.0 ± 5.6	39.6 ± 12.7	78.1 ± 16.8
144	19.8 ± 21.8	19.0 ± 2.6	24.5 ± 3.5	35.5 ± 7.4

TABLE 2

DETECTABLE DIPHENADIONE RESIDUES (> 0.01 ppm) IN TISSUES OF CATTLE GIVEN SINGLE 1 mg/kg INTRARUMINAL INJECTIONS

Days posttreatment	ppm found (mean ± SD)[a]	
	Liver	Kidney
30	0.15 ± 0.01	0.08 ± 0.01
60	0.14 ± 0.01	0.10 ± 0.02
90	0.15 ± 0.00	0.08 ± 0.00

[a] Means of four values (two animals at each period run in duplicate).

Efficacy of the Method

Thompson *et al.* (1972) reported that one systemic treatment of cattle on three ranches in Mexico reduced the number of vampire bat bites by 93 percent. Immediately before treatment, 214 bites were counted on 207 cattle; 2 weeks after treatment, 15 bites were counted on 210 cattle. According to a prediction equation derived earlier, the population had been reduced from 212 to 15 vampire bats (Thompson *et. al.*, 1972). Similar results were obtained in another study on 58 dairy cows in Ecuador and 28 cows in Nicaragua. In Ecuador vampire bat bites averaged 3.95 per cow before treatment and only 0.07 after 60 days; in Nicaragua bites averaging 2.61 per cow before treatment were completely eliminated (Thompson and Elias, in review).

Nicaragua's National Campaign

Some of the best data on the practical application of the systemic method has come from Nicaragua. In 1974 this country began a National Campaign to control vampire bat parasitism on livestock (González and Mitchell, 1976). The board of Livestock Services and Control administers the program through a staff of trained professionals. The program chief and field supervisors are veterinarians and the field teams are personnel trained in general agriculture and vampire bat control methodology. Thus far the teams have conducted bat control in 9 of the 17 departments (states) of the country.

Large numbers of cattle have been treated by the systemic method. For example, on 18 ranches in the departments of Granada and Rivas, where the topical method was not used, 18,714 cattle were treated and vampire bites were reduced by 93.7 percent.

In the total campaign 102,349 cattle have been treated systemically and 1,488 vampire bats treated topically. Examinations 30 days after treatment have shown that biting has been reduced by 91.8 percent overall (González and Mitchell, 1976). Dr. Rodrigo González, Director General of Livestock in the Ministry of Agriculture, has stated that no confirmed cases of vampire bat-transmitted rabies have been reported in Nicaragua since 1974 and that the Ministry of Agriculture believes the problem has been eliminated as a result of the control campaign (González and Mitchell, 1976).

Human Safety - Analysis of Residues

After developing the systemic method of vampire control and finding it safe for adult cattle and sheep, we shifted our research emphasis to aspects of human safety. The important question was to determine if there would be a hazard to humans if they consumed meat and milk from livestock shortly after the animals were treated. For these studies, a method was developed to chemically analyze the concentration of diphenadione in blood plasma, milk, and animal tissues (Bullard *et al.*, 1975).

Tissue Residues in Cattle

In a tissue residue study (Bullard *et al.*, 1976), six Hereford heifers weighing approximately 230 kg each were injected intraruminally with 1 mg/kg of diphenadione. Two of the animals were then killed at 30, two at 60, and two at 90 days post-treatment. Samples of each animal's blood plasma, liver, heart, kidney, brain, hindquarter muscle, and fat (1:1 mixture visceral and subcutaneous) were analyzed for diphenadione residues. Detectable levels (0.01 ppm or more) were found in kidney and liver samples but not in the other tissues (Table 2).

The range of daily adult single oral dosages for diphenadione as a prothrombinopenic anticoagulant in human therapy is 2.5 to 30 mg (Remington's Pharmaceutical, 1970). If liver contained the highest concentration found (0.15 ppm), a human would have to eat nearly 17 kg of it per day to receive the minimum daily dosage used in anticoagulant therapy.

TABLE 3

DIPHENADIONE RESIDUES IN THE MILK OF COWS, AND PLASMA PROTHROMBIN CLOTTING TIMES OF THE COWS AND THEIR NURSLING CALVES, AT INTERVALS AFTER THE COWS WERE GIVEN SINGLE INTRARUMINAL INJECTIONS OF DIPHENADIONE

	Control (1 cow and calf)			1 mg/kg (3 cows and calves)			2.75 mg/kg (3 cows and calves)		
Hours post-treatment	ppm diphenadione in milk[a]	Prothrombin clotting time in sec Cow	Prothrombin clotting time in sec Calf	ppm diphenadione in milk[a]	Prothrombin clotting time in sec (mean±SD) Cows	Prothrombin clotting time in sec (mean±SD) Calves	ppm diphenadione in milk (mean±SD)[a]	Prothrombin clotting time in sec (mean±SD) Cows	Prothrombin clotting time in sec (mean±SD) Calves
0	ND[b]	19.0	18.0	ND	15.3±0.3	14.8±0.7	ND	16.7± 1.4	17.3±0.6
12	ND	17.0	15.7	ND	18.7±2.5	16.7±1.6	0.019±0.007	16.7± 0.4	15.5±0.2
24	ND	16.5	15.4	ND	19.0±2.2	15.8±0.3	0.021±0.005	22.0± 0.9	15.9±0.1
48	ND	19.0	17.0	ND	25.3±4.2	18.5±1.6	0.020±0.007	29.2± 2.6	16.0±1.3
72	ND	15.8	15.4	ND	24.6±6.8	15.2±0.2	ND	39.8± 4.5	15.3±0.6
96	ND	16.1	15.5	ND	21.2±4.4	15.7±0.8	ND	42.0± 8.0	15.7±0.6
120	ND	16.8	16.0	ND	20.3±2.8	16.3±0.2	ND	30.5±10.6	15.2±0.2
144	ND	15.0	15.5	ND	16.7±2.5	14.4±1.2	ND	23.2± 8.0	14.3±0.6

[a] All milk samples, from each animal at each interval, run in duplicate.

[b] ND = not detected (limit of detection, 0.003 ppm).

In addition, the livers of the six treated heifers were used in a secondary hazard study with rats, which are extremely sensitive to multiple doses of diphenadione (chronic LD_{50}, 0.2 mg/kg/day). Liver from each treated heifer was fed to 10 adult Sprague Dawley rats for 14 days; each rat received a daily ration equivalent to 1.36 kg for a 70 kg human. Ten rats fed untreated liver served as controls. All rats ate all liver offered and all gained weight. At 14 days, 5 of each 10 rats were killed, their blood was immediately analyzed for prothrombin clotting times, and blood and liver samples were taken and analyzed for diphenadione residues. The remaining rats in each group were observed for an additional 1[illegible] days for mortality or signs of chronic toxicity. None of the rats showed any ill effects throughout the study. There was no difference in prothrombin clotting times among treated or control groups, and diphenadione was not detected in the liver or blood from treated rats.

These results were corroborated by an earlier secondary hazard study conducted at a higher dosage level (Thompson *et al.*, 1972). The livers of two beef calves slaughtered at 30 days after an oral dose of 2.75 mg/kg were fed to 30 laboratory rats for 7 consecutive days. Each day the rats consumed an equivalent of 6.8 kg per 70 kg man with no signs of intoxication or change in prothrombin clotting times. The fact that the tissue of greatest diphenadione concentration, from an animal given 2.75 times the recommended dosage, had no effect on rats is further evidence of the safety of the method.

Residues in Milk of Cattle

In the milk residue study (Bullard *et al.*, 1977), three lactating cows were injected intraruminally with 1 mg/kg doses of diphenadione, three others were injected with 2.75 mg/kg, and a control cow received a "shara" injection of physiological saline. Each cow had a nursling calf. Samples of milk and blood from each cow and blood from each calf were taken at 0, 12, 24, 48, 72, 96, 120, and 144 hours post-treatment and analyzed for diphenadione residues. Prothrombin clotting times were determined on all blood samples immediately after collection.

Diphenadione could not be detected in any blood samples from nursling calves. Traces of diphenadione were found in milk samples taken at 12, 24, and 48 hours post-treatment from cows given 2.75 mg/kg but could not be detected in milk from cows given the recommended 1 mg/kg doses (Table 3). However, even the trace residues for the 2.75 mg/kg dose did not appear in the blood of the nursling calves and there were no detectable increases in prothrombin clotting times (Table 3).

Even if milk contained the 0.021 ppm maximum residue level resulting from accidentally administering 2.75 times the recommended dosage, a person would have to drink 117 liters of it to obtain the 2.5 mg minimum daily dosage used in human anticoagulant therapy. Since detectable quantities of diphenadione did not occur in the milk of cows given the recommended 1 mg/kg dosage, the method's safety seems assured.

Conclusion

The systemic method of vampire bat control has been tested and found to be highly effective in studies in Mexico, Ecuador, and Nicaragua. Futhermore, it has been the predominant method used in the National Campaign in Nicaragua, where bat-transmitted rabies appears to have been eliminated as a result. Since there have been no reported ill effects in any of the adult cattle treated in any of the countries and our investigations indicate that diphenadione residues do not pose a hazard to the health of either livestock or man, a safe and effective method of vampire bat control is available for use by the countries of Latin America.

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NOTES

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EFICACIA Y SEGURIDAD DEL METODO SISTEMICO EN EL CONTROL DEL MURCIELAGO VAMPIRO

ROGER W. BULLARD
Y R. DANIEL THOMPSON

RESUMEN *En el método sistémico para controlar murciélagos vampiros* (Desmodus rotundus), *se suministra al ganado una sola inyección de 1 mg/kg de difenadiona (2-difenilacetil-1,3 indandiona). Este anticoagulante es rápidamente absorbido por el flujo sanguíneo y cualquier murcielago que se alimente de un animal que ha recibido este tratamiento, en 72 horas recibe una dosis letal. Esta inyección no es dañina para el ganado adulto, pues éste recibe abundante vitamina K (antídoto) de la bacteria de su rumen y también de plantas verdes. Por otra parte, los murciélagos vampiros son sumamente susceptibles a este anticoagulante. La dosis por vía oral calculada estadísticamente como mortal para un 50 por ciento (DL_{50}) de los murciélagos inactivos tratados es de 0.91 mg/kg y, posiblemente, un tercio de este nivel para murciélagos en movimiento. En pruebas de campo realizadas en México, Ecuador y Nicaragua este método sistémico ha demostrado ser muy efectivo y seguro. También ha sido empleado en forma extensiva en una campaña nacional en Nicaragua, donde los ataques por murciélagos vampiros han sido reducidos en más de un 90 por ciento y la rabia transmitida por ellos parece haber sido eliminada.*

Estudios adicionales han demostrado que los residuos de difenadiona en la carne y leche del ganado tratado no presentan riesgo para la salud humana. No se percibieron residuos en la leche de vacas tratadas con la dosis recomendada de 1 mg/kg, pero en vacas dosificadas al 2.75 mg/kg residuos de 0.021 ppm o inferiores fueron encontrados. Solamente se percibió difenadiona en muestras de hígado y riñón (0.15 ppm o inferiores) 30 días después de que se le administró al ganado una dosis de 1 mg/kg Por 2 a 4 semanas se alimentaron ratas con hígado de ganado al que se le había administrado el tratamiento, y estas ratas no mostraron efectos adversos. Estos niveles de residuos están por debajo de la dosis mínima diaria de difenadiona usada en la terapia humana.

EFICÁCIA E SEGURANÇA DO MÉTODO SISTÊMICO NO CONTROLE DO MORCEGO VAMPIRO

ROGER W. BULLARD
E R. DANIEL THOMPSON

RESUMO *No método sistêmico para controlar morcegos vampiros* (Desmodus rotundus), *faz-se uma única injeção intraruminal de 1 mg/kg de difenadiona (2-difenilacetil-1,3 indadiona). Este anticoagulante é rápidamente absorvido pela corrente sanguínea e qualquer morcego que, no intervalo de 72 horas, venha a alimentar-se de um animal que tenha recebido tal tratamento recebe uma dose letal. Esta injeção é inócua para o gado adulto, pois este recebe abundante antídoto na vitamina K produzida pelas bactérias de seu rumen e também contida em plantas verdes. Por outro lado, os morcegos vampiros são sumamente suscepíveis a este anticoagulante: a dose letal 50 por cento (LD_{50}) dos morcegos inativos tratados é de 0,91 mg/kg e, posteriormente, um terço deste nível para morcegos em atividade. Em provas efetuadas no campo, no México, Equador e Nicaragua este método sistêmico tem demonstrado ser bastante efetivo e seguro. Também foi empregado em forma extensiva em uma campanha nacional em Nicaragua, onde os ataques por morcegos vampiros estão se reduzindo em mais de 90 por cento e a raiva transmitida por morcegos parece ter sido eliminada.*

Estudos adicionais demonstraram que os resíduos de difenadiona na carne e no leite do gado tratado não apresentam riscos para a saúde humana. Não foram percebidos resíduos no leite das vacas tratadas com a dose recomendada de 1 mg/kg, porém em vacas que receberam 2,75 mg/kg acharam-se resíduos de 0,021 ppm ou menos. Somente se percebeu difenadiona em amostras de fígado e de rins (0.15ppm ou menos) 30 dias depois de ser administrada ao gado uma dose de 1 mg/kg. Durante 2 a 4 semanas alimentaram-se ratos com fígado de gado submetido ao tratamento, e estes ratos não mostraram efeitos adversos. Estes níveis de resíduos são pequenos em comparação com a dose mínima diária de difenadiona usada na terapia humana.