INTRAMUSCULAR INOCULATION OF CATTLE WITH WARFARIN: A NEW TECHNIQUE FOR CONTROL OF VAMPIRE BATS

Raúl Flores Crespo,2 Salvador Said Fernández,3 Donaciano De Anda López,4 Froylán Ibarra Velarde,5 Rosa María Anaya D.G.6

Approximately one-half million head of cattle are estimated to die annually in Latin America from rabies transmitted by vampire bats. This article describes the development and testing of a new vampire bat control method that appears to offer important advantages over other available techniques.

Introduction

A number of Latin American countries have recently implemented new techniques for controlling blood-drinking vampire bats, the principal transmitters of paralytic rabies to cattle. Developed over the past decade, these techniques use anticoagulants to kill the bats.

One procedure involves capturing a small number of vampires and smearing their backs with some 1.5 cc of vaseline containing a suspended anticoagulant—either chlorophacinone [2-(p-phenylacetyl) phenylacetyl-1,3-indandione] or diphenadione (2-diphenylacetyl-1,3-indandione). The treated vampires are then released to return to their roosts, where other members of the colony groom them, a process involving licking, and thereby ingest a fatal dose of anticoagulant (1).

Another procedure calls for applying chlorophacinone or warfarin [9-(α-acetonylbenzyl)-4-hydroxycoumarin] suspended in vaseline directly to the surface of the niches in the vampires' roosts (2, 3). A third technique is to apply vaseline containing warfarin to vampire bite marks on cattle (3)—an approach based on the fact that the vampires generally return to reopen previously inflicted wounds (4). Finally, a fourth technique consists of injecting diphenadione suspended in Carbopol 9517 into the rumens of vulnerable cattle (5).

While all of these techniques have proved effective, all of them also have drawbacks that limit their applicability in the field. For instance, the first procedure described requires deployment of specialized personnel and equipment. The second has the same requirement and also necessitates entering vampire roosts. The third does not demand specialized personnel and equipment but requires frequent cattle handling, a condition posing a serious constraint. And the fourth procedure, besides demanding

1Also appearing in Spanish in the Boletín de la Oficina Sanitaria Panamericana, 1979. Partial results of this work were presented in an address at the First Symposium on Hematophagous Bats and Problems Associated with Paralytic Rabies held at Managua, Nicaragua, on 28-30 June 1976; and a summary of this work was published in the abstracts of the Symposium on Advances in Rabies Research held at the Center for Disease Control in Atlanta, Georgia, on 7-9 September 1976.

2Biologist; Chief of the Vampire Bat Control Program, National Institute of Livestock Research (INIP), Mexico City, Mexico.

3Veterinarian, INIP Vampire Bat Control Program. Currently engaged in fellowship work at the Division of Advanced Studies of the National Polytechnic Institute in Mexico City.

4Veterinarian; Researcher, INIP Vampire Bat Control Program, Mexico City.

5Veterinarian, INIP Vampire Bat Control Program, Mexico City.

6Chemist; Researcher, INIP Vampire Bat Control Program, Mexico City.

7A suspending agent available from B. F. Goodrich.
enough technical and anatomical knowledge to inject anticoagulant into bovine rumens is only effective for the relatively short time that adequate levels of anticoagulant are circulating in the blood; moreover, because of the risk of intoxication this procedure cannot be applied to cattle under 1 year of age.

In view of these problems, the vampire bat control project at the Mexican Ministry of Agriculture and Water Resources' National Livestock Institute has carried out a series of studies for the purpose of developing a control method that would not be subject to such limitations. The object of this article is to report the results of those studies.

The active principle used in this investigation was warfarin, which blocks the action of vitamin K, inhibiting the mechanism of coagulation, and also weakens the vascular endothelia, especially of the capillaries, thereby inducing internal bleeding. Since the common vampire bat (*Desmodus rotundus*), like most rodents, has a very low body weight (30 grams on the average) and a very rapid basal metabolism relative to the body weights and basal metabolisms of cattle, it was felt that a small dose of warfarin injected into cattle and circulating in their blood could prove sufficient to kill feeding vampires without endangering the cattle's health. The intramuscularly injectable compound employed in the studies was prepared in an aqueous solution at a concentration of 100 mg/ml and called "Vampirinip III." 8

The immediate goals of the work described were as follows: (1) to determine the vampiricidal potency of warfarin injected intramuscularly into cattle under laboratory conditions; (2) to study the pharmacologic and toxicologic effects of the injected material on cattle of various breeds, ages, and stages of physiologic development; and (3) to evaluate the efficacy of the proposed vampire control method under field conditions.

8Vampirinip III is the third vampiricide developed by INIP. Vampirinip I was applied topically directly to the vampires, and II was used topically in vampires and/or the fresh bites in cattle. Product registration is in progress.
Potency of Vampirinip III under Laboratory Conditions

For the initial laboratory tests the following procedure was adopted: A corral was built indoors. This corral, 2.5 meters high and 6 meters on a side, was made vampire-tight with iron bars and wire netting and was divided into two equal but separate vampire-tight compartments designated A and B. An artificial vampire roost with optimum temperature (24°C) and relative humidity (above 50 per cent) was installed in the upper part of each compartment. These conditions were maintained in each roost by keeping water dripping there and by installing thermostatically regulated electric heaters.

A colony of vampire bats (*Desmodus rotundus*) was then placed in compartment A. Each bat was identified by a numbered metal tag attached to the leading edge of one wing. The door of roost A was opened at dusk to allow the colony of vampires to feed on two cattle that were herded into compartment A each afternoon and kept there overnight. Once the vampires adapted to these conditions and returned regularly to the artificial roost of their own volition after feeding, the laboratory tests were begun.

Three experiments were carried out to test the effects of the warfarin in Vampirinip III at different strengths.

Twenty vampires were used in the first test. On the first morning of this test Vampirinip III was injected intramuscularly into two adult cattle at a concentration of 2 mg warfarin per kg of body weight. These cattle were then led into compartment B, and four of the 20 vampires were placed in the compartment B roost. At dusk, 8 hours after the cattle were treated, the gate of the roost was opened and the four bats were allowed to feed on the treated cattle. The next morning these vampires were returned to the compartment A roost, and another group of four vampires was placed in the compartment B roost. This procedure was repeated daily until five groups of four vampires (20 bats in all) had been exposed. In this manner the bats in each lot were exposed to the treated compartment B cattle only one night, before and after which they fed on the untreated cattle in compartment A.

The second experiment followed the same pattern, except that 4 mg/kg were injected, 16 vampire bats were used instead of 20, and the test ran 4 instead of 5 days. The third experiment, like the first, ran 5 days; 6 mg/kg were injected; and 20 bats were employed.

The results obtained are shown in Table 1. At a strength of 2 mg/kg, 100 per cent mortality occurred only among those bats exposed the first night after the cattle were treated. Only half the bats died that were exposed on the second, third, and fourth nights; and only one of the bats exposed the fifth night perished. At a strength of 4 mg/kg, mortality was 100 per cent the first and second nights, 75 per cent the third
Table 1. Mortality in three colonies of captive vampire bats exposed to cattle treated with Vampirinip III.

| Dose of active principle received by each injected bovine, per kg of body weight | Mortality (%) among groups of 4 bats exposed to treated cattle on each of the first 5 nights following treatment |
|---|---|---|---|---|---|
| Night 1 | Night 2 | Night 3 | Night 4 | Night 5 |
| 2 mg | 100 | 50 | 50 | 50 | 25 |
| 4 mg | 100 | 100 | 75 | 50 | – |
| 6 mg | 100 | 100 | 100 | 100 | 50 |

night, and 50 per cent the fourth night. Finally, at a strength of 6 mg/kg, mortality was 100 per cent on the first four nights, declining only on the fifth night to 50 per cent.

Autopsies performed on the dead vampires revealed typical symptoms of anticoagulant poisoning. External examination showed hemorrhaging along the base of the toenails, in the soft parts of the thumbs, in the nostrils, and in the genital orifices, as well as severe hematomas on the arms and wing membranes and around the interfemoral membrane. Internally there was considerable congestion of the meninges, spinal bulb, and cerebral cortex, with hemorrhages and hematomas appearing in various bodily tissues and organs; a pale liver and uncoagulated blood in the ventricles of the heart were observed in all cases.

Effects of Vampirinip III on Cattle

A number of experiments were designed in order to evaluate the pharmacologic and toxicologic effects of Vampirinip III on cattle, giving particular attention to hematologic and physiological indicators—especially prothrombin times; to the safety margins involved; and to the process of anticoagulant elimination.

1. Hematologic Testing of Treated Cattle

The aim of this test was to determine possible changes in the hematology of animals treated with Vampirinip III. The animals tested were eight Indobrasil calves of similar weight that were approximately eight months old. The group was divided randomly into two lots. An intramuscular injection of Vampirinip III (a dose containing 6 mg warfarin per kg of body weight) was administered to the cows in lot 1. Lot 2 served as a control group. Blood was taken daily from the jugular veins of the calves in both lots for 10 days—the five days preceding and following treatment of lot 1. Physiological indicators (rectal temperature, heart rate, and respiration rate) were measured daily. The blood samples obtained were subjected to hematologic biometry, and determinations were made of hematocrits, prothrombin times, and whole blood coagulation times.

The results indicated that the treated calves' rectal temperatures, heart rates, and respiration rates remained within normal limits throughout the experiment, being virtually identical to those of the control group.

Hematologic biometry findings were as follows: For all cattle in both treated and control groups, leukocyte counts were within the range (4,900-19,500 per mm$^3$) reported by Benjamin (6) as normal for young cattle; erythrocyte counts were within the range (6,080,000-13,800,000 per mm$^3$) reported by Miller as normal; and hemoglobin levels were within the range (8.7-14.5 g per 100 ml) reported by Albritten (8) as normal.
Regarding prothrombin times, a lag was observed in the treated groups 24 hours (1 day) after the administration of Vampirinip III. The maximum lag—indicating an 84 per cent decline in prothrombin activity—was found at 72 hours (3 days), after which the lag gradually diminished, prothrombin times again reaching normal levels after 168 hours (7 days). No change in prothrombin times was observed among cattle in the control group.

A similar pattern was observed with respect to whole blood coagulation times, the maximum lags occurring at 3 days, with a return to normal levels at 7 days.

2. Vampirinip III in Milk from Treated Cows: Effect on Calf Prothrombin Times

This test sought to determine whether dairy cows injected with Vampirinip III would eliminate sufficient warfarin in their milk to alter the prothrombin times of calves drinking that milk. The following procedure was used: Two lots of cattle were selected, one consisting of 10 lactating Holstein cows weighing between 250 and 350 kg, the other composed of 10 Holstein calves approximately 15 days old. All the milk obtained from the cows was used to feed the calves, and all the milk from a particular cow was given daily in a pail to the same calf for the whole duration of the experiment. Blood was taken daily from the jugular veins of all cows and calves in order to measure prothrombin times. On the fifth day of the test, after the taking of blood samples, Vampirinip III (5 mg warfarin per kg of body weight) was injected into each of the 10 adult cows. The taking of daily blood samples continued, and prothrombin times were measured using the Sera Tek (Ames Laboratories) reaction chambers technique.

Figure 1 shows the average prothrombin times of both cows and calves over the course of the 22-day experiment. The cows' average prothrombin times were 23.3 ± 2.1 seconds before treatment, 29.1 seconds the day following treatment, and 46.3 seconds the third day following treatment. After reaching this latter peak the average prothrombin time declined, returning to its initial value on the seventh day follow-

Figure 1. Prothrombin times of cows treated with Vampirinip III (5 mg warfarin per kg body weight) and of calves fed on their milk.
ing treatment. The maximum value recorded (46.3 seconds) represented a 61.3 percent reduction in prothrombin activity. For the calves being fed on these cows' milk, the average prothrombin time before the cows were treated was 24.8 ± 3.1 seconds. As indicated in Figure 1, treatment of the cows produced no significant change in this value.

3. Vampirinip III Administered to Pregnant Cows and Calves: Effect on Prothrombin Times

To provide a basis for deciding which cattle should or should not be treated, an experiment designed to assess changes in physiological indicators and prothrombin times of treated calves and pregnant cows was carried out. As in the forementioned test, prothrombin times were determined using the Sera Tek reaction chambers technique.

Four lots of cattle were selected. Lot 1 consisted of four cows in the last third of gestation; lot 2 consisted of four cows in the first third of gestation; lot 3 consisted of four cows approximately 1 year old that had never been inseminated; and lot 4 consisted of calves approximately 40 days old. A sample of blood was taken daily from the jugular vein of each animal for determination of prothrombin times. On the fifth day, after taking blood, Vampirinip III (containing a dose of 5 mg warfarin per kg body weight) was injected into all the animals. The taking of daily blood samples continued; rectal temperatures, heart rates, and respiration rates were recorded daily throughout the experiment.

The physiological indicators showed no significant changes, either in the five days before treatment or on subsequent days, remaining at virtually the same levels throughout the test. The average respective values for lots 1, 2, 3, and 4 were as follows:

Figure 2. Prothrombin times of pregnant cows, yearling heifers, and 40-day-old calves injected with Vampirinip III (5 mg warfarin per kg body weight).
temperatures—38.7°C, 38.5°C, 38.6°C, and 39.2°C; heart rates—72, 71, 69, and 123; and respiration rates—33, 31, 36, and 43. These values are within the normal limits reported by Dukes (9). With regard to individual cattle, none of the values recorded exceeded normal limits.

Regarding prothrombin times, the average values obtained for each lot of cattle are shown in Figure 2. As may be observed, these values remained constant for all the lots during the five days preceding treatment. Subsequently, beginning on the sixth day (i.e., 24 hours after treatment), each lot showed a delay in prothrombin time that reached a maximum value on the third day after treatment. This was followed by a gradual decline, initial levels being reached on the ninth or tenth days after treatment (days 14 or 15 on the chart). As indicated, the smallest prothrombin time changes were experienced by the pregnant cows in lots 1 and 2, the observed changes being similar for both lots. Prothrombin times for lot 3 (1-year-old heifers) yielded a curve whose values were within acceptable limits for anticoagulant therapy. Lot 4 (40-day-old calves) showed marked prothrombin time lags, probably because young calves normally have a physiological vitamin K deficiency resulting from scanty or slight development of intestinal and ruminal flora at this age. However, the level of prothrombin activity only declined to 75 per cent below normal, which is still higher than the level indicated by Goodman and Gilman (10) as adequate in anticoagulant therapy.

### 4. Physiological Indicators in Cattle Treated with Vampirinip III

Another experiment sought to assess changes in physiological indicators (rectal temperature, heart rate, and respiration rate) among adult cattle injected with progressively larger doses of Vampirinip III. For this purpose 30 male animals ranging from 1 to 5 years old and weighing anywhere from 100 to 400 kg were selected. (The group included Brahman, Hereford, Indobrasil, Brown Swiss, and Zebu x Brown Swiss crosses.) The animals were divided at random into six lots of five. Lot 1 served as a control group. The lot 2 animals received a dose (5 mg warfarin per kg) shown to be appropriate by previous studies; then, 24 hours later, their horns were removed to see whether trauma of this kind would cause hemorrhage problems. The cattle in lots 3, 4, and 5 received 6, 8, and 10 mg of warfarin per kg, respectively. All the cattle were brought in twice daily, in the morning and afternoon, for the purpose of determining their temperatures, heart rates, and respiration rates.

The results of this test—with regard to average daily values and overall averages for each lot—are shown in Tables 2, 3, and 4. As may be seen, the values obtained for lot 1 were similar to those obtained for the

<table>
<thead>
<tr>
<th>Day</th>
<th>Lot 1 (controls, 0 mg/kg)</th>
<th>Lot 2 (dehorned, 5 mg/kg)</th>
<th>Lot 3 (6 mg/kg)</th>
<th>Lot 4 (8 mg/kg)</th>
<th>Lot 5 (10 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>39.3°</td>
<td>39.2°</td>
<td>39.7°</td>
<td>39.6°</td>
<td>39.6°</td>
</tr>
<tr>
<td>2</td>
<td>38.7°</td>
<td>38.3°</td>
<td>39.1°</td>
<td>38.8°</td>
<td>38.9°</td>
</tr>
<tr>
<td>3</td>
<td>38.5°</td>
<td>38.5°</td>
<td>39.0°</td>
<td>38.7°</td>
<td>39.0°</td>
</tr>
<tr>
<td>4</td>
<td>38.8°</td>
<td>38.9°</td>
<td>39.2°</td>
<td>38.9°</td>
<td>39.2°</td>
</tr>
<tr>
<td>5</td>
<td>38.6°</td>
<td>38.8°</td>
<td>38.9°</td>
<td>38.8°</td>
<td>38.9°</td>
</tr>
<tr>
<td>6</td>
<td>38.5°</td>
<td>38.1°</td>
<td>38.6°</td>
<td>38.4°</td>
<td>38.8°</td>
</tr>
<tr>
<td>6-day average</td>
<td>38.7°</td>
<td>38.6°</td>
<td>39.1°</td>
<td>38.9°</td>
<td>39.1°</td>
</tr>
</tbody>
</table>
Table 3. Average respiration rates among 5 groups of cattle, following treatment with Vampirinip III.

<table>
<thead>
<tr>
<th>Day</th>
<th>Lot 1 (controls, 0 mg/kg)</th>
<th>Lot 2 (dehorned, 5 mg/kg)</th>
<th>Lot 3 (6 mg/kg)</th>
<th>Lot 4 (8 mg/kg)</th>
<th>Lot 5 (10 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (treatment)</td>
<td>44.4</td>
<td>40.0</td>
<td>51.9</td>
<td>48.8</td>
<td>48.3</td>
</tr>
<tr>
<td>2</td>
<td>39.2</td>
<td>41.5</td>
<td>47.6</td>
<td>46.6</td>
<td>41.6</td>
</tr>
<tr>
<td>3</td>
<td>38.8</td>
<td>40.5</td>
<td>45.3</td>
<td>45.3</td>
<td>45.3</td>
</tr>
<tr>
<td>4</td>
<td>37.2</td>
<td>36.8</td>
<td>40.3</td>
<td>39.3</td>
<td>40.0</td>
</tr>
<tr>
<td>5</td>
<td>34.0</td>
<td>33.9</td>
<td>35.6</td>
<td>36.3</td>
<td>36.0</td>
</tr>
<tr>
<td>6</td>
<td>38.0</td>
<td>28.6</td>
<td>36.3</td>
<td>32.9</td>
<td>34.3</td>
</tr>
<tr>
<td>6-day average</td>
<td>38.6</td>
<td>37.1</td>
<td>42.8</td>
<td>41.5</td>
<td>40.9</td>
</tr>
</tbody>
</table>

Table 4. Average heart rates among 5 groups of cattle, following treatment with Vampirinip III.

<table>
<thead>
<tr>
<th>Day</th>
<th>Lot 1 (controls, 0 mg/kg)</th>
<th>Lot 2 (dehorned, 5 mg/kg)</th>
<th>Lot 3 (6 mg/kg)</th>
<th>Lot 4 (8 mg/kg)</th>
<th>Lot 5 (10 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (treatment)</td>
<td>111.9</td>
<td>100.3</td>
<td>113.9</td>
<td>118.0</td>
<td>120.1</td>
</tr>
<tr>
<td>2</td>
<td>96.4</td>
<td>101.5</td>
<td>112.0</td>
<td>98.0</td>
<td>100.6</td>
</tr>
<tr>
<td>3</td>
<td>85.6</td>
<td>91.6</td>
<td>99.1</td>
<td>95.3</td>
<td>100.1</td>
</tr>
<tr>
<td>4</td>
<td>79.6</td>
<td>86.0</td>
<td>85.6</td>
<td>88.3</td>
<td>84.8</td>
</tr>
<tr>
<td>5</td>
<td>73.6</td>
<td>75.9</td>
<td>77.6</td>
<td>81.6</td>
<td>79.9</td>
</tr>
<tr>
<td>6</td>
<td>69.2</td>
<td>72.4</td>
<td>81.3</td>
<td>75.9</td>
<td>79.9</td>
</tr>
<tr>
<td>6-day average</td>
<td>86.1</td>
<td>87.9</td>
<td>95.0</td>
<td>92.5</td>
<td>94.3</td>
</tr>
</tbody>
</table>

Other lots. Specifically, no significant difference was observed between the indicator values obtained for lot 1 (the control group), lot 2 (the dehorned animals), or lot 5 (animals receiving twice the recommended dose). Heart and respiration rates tended to be somewhat high, even in the control group, especially in the first days of the experiment. This was probably because the test animals in the experiment had just been brought in from pasture and were not accustomed to handling. However, in all cases the values shown in the tables are within the normal limits cited by Duke (9).

5. Warfarin Levels in Milk and Tissues of Treated Cattle

Another experiment employed radioactive warfarin (containing carbon 14) in order to quantitatively assess the possible risk to people consuming meat or milk from Vampirinip-treated cattle.

The following procedure was used: Four milk-cows were given separate intramuscular injections of radioactive warfarin (5 mg/kg). Each treated cow was then placed in an enclosure with concrete walls and floor—a sort of metabolic cage. Specimens of accumulated feces and urine were collected every 4 hours in daytime and at the end of a 12-hour nighttime period. Milk samples were taken twice daily—at the regular 8:00 a.m. and 5:00 p.m. milkings. Two of the cows were sacrificed at 120 hours and the other two at 384 hours after treatment. Specimens of each cow's liver, kidneys, spleen, pancreas, lungs, heart, and skeletal muscles were examined. Radioactivity levels were determined by liquid scintillation (Packard spectrometer Tricarb 3330, and Instagel scintillation fluid).
The results showed that the radioactive material was primarily excreted in urine and feces: In the two cows slaughtered at 120 hours after treatment, excretion in urine was 68.0 and 60.5 per cent and in feces 18.3 and 10.8 per cent. In the two cows slaughtered at 384 hours, excretion in urine was 63.5 and 67.0 per cent and in feces 18.3 and 17.8 per cent. Only 0.1 per cent of the radioactive material injected was eliminated in milk. We assume, moreover, that this 0.1 per cent did not emerge in the form of warfarin but as two more polar compounds—as indicated by low paper chromatography values (obtained in tests using Whatman No. 1 paper) measured against a warfarin standard. Even if the warfarin had remained unchanged, however, the highest levels found in the milk of cows 1, 2, 3, and 4, respectively (see Table 5) would have been 0.15, 0.05, 0.098, and 0.125 parts of warfarin, by weight, per million parts of milk.

The radioactivity of each tissue examined, in terms of parts of warfarin, by weight, per million parts of sample, assuming no metabolic breakdown, is shown in Table 5. As may be seen, the levels of radioactivity varied considerably—presumably because the cows were sacrificed at different times and also because the injected solution’s variable pH—which was not controlled in this study—permitted variable absorption. In the case of the second cow, the time required to eliminate half the absorbed material was very long. This is reflected in the levels of radioactivity found in the tissues—levels far higher than those observed in the first cow’s tissues even though both animals were slaughtered at 120 hours. Smaller differences were observed in the radioactivity levels of tissues from cows 3 and 4 sacrificed at 384 hours. These radioactivity levels were also lower than those found in the tissues of cows 1 and 2, because cows 3 and 4 were able to eliminate the drug for a much longer time. As Table 5 indicates, values cited are overall radioactivity levels, the minimal levels found making it impractical to try and distinguish between warfarin and its metabolic by-products. One of the highest radioactivity levels found, 7.12 ppm, was detected in the liver of cow 2. In contrast, the highest level detected in milk was 0.15 ppm in cow 1.

Field Tests

The encouraging results of the foregoing tests prompted a decision to evaluate the proposed bat control method under field conditions. The following ranches were selected for this purpose: “San Carlos” and “San Pedro,” both in the common border area of Tixkocob and Tixpeual, two towns in the state of Yucatán; “Kanto” in the Yucatán township of Valladolid; and

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Cow 1 (120 hrs.)</th>
<th>Cow 2 (120 hrs.)</th>
<th>Cow 3 (384 hrs.)</th>
<th>Cow 4 (384 hrs.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>2.21</td>
<td>7.12</td>
<td>1.74</td>
<td>1.91</td>
</tr>
<tr>
<td>Kidney</td>
<td>1.20</td>
<td>2.76</td>
<td>0.68</td>
<td>0.49</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.80</td>
<td>2.54</td>
<td>—^a</td>
<td>0.31</td>
</tr>
<tr>
<td>Pancreas</td>
<td>0.39</td>
<td>8.68</td>
<td>2.20</td>
<td>1.48</td>
</tr>
<tr>
<td>Lung</td>
<td>0.09</td>
<td>1.59</td>
<td>0.25</td>
<td>0.11</td>
</tr>
<tr>
<td>Heart</td>
<td>0.27</td>
<td>1.04</td>
<td>0.15</td>
<td>0.05</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>0.17</td>
<td>0.03</td>
<td>0.15</td>
<td>0.06</td>
</tr>
</tbody>
</table>

^aNo radioactivity detected.
"Ojo de Agua" of Huichihuayan, a township in the state of San Luis Potosí. For many years the cattle at all four ranches had been subjected to high rates of vampire bat attack.

The same procedure was followed at each ranch. Early in the morning the cattle were examined, and a record was made of each animal's weight, breed, and number of visible bites inflicted by vampire bats. At San Carlos 49 Brahman cattle were rounded up, but 9 escaped from the chute and could not be examined. Of the 40 examined, 7 appeared free of vampire wounds while the remaining 33 exhibited evidence of 81 recent bites. At San Pedro 25 Holstein cattle were examined. All had been bitten by vampires, there being evidence of 52 recent bites. At Kanto, where there were about 500 head of Zebu-crossed native cattle, only 392 could be delivered to the chute for examination. All but 90 of these showed evidence of recent bites, the total bite count being 890. At Ojo de Agua 40 Swiss and Zebu-crossed Swiss cattle were examined. Only two were found free of bite marks, 178 recent bites being counted on the other 38.

The treatment applied at all the ranches was to inject Vampirinip III (5 mg/kg) into each animal showing evidence of recent vampire bites. This was done at the time the bites were counted. The weights of the treated animals varied widely—from 80-100 kg for the smallest to over 400 kg for the largest. In all cases the examined and treated cattle were marked in order to avoid errors during subsequent reexamination.

Somewhere between 14 and 17 days after treatment, a second visit was made to each ranch and the vampire bite marks were recounted. In all cases the evaluation was made the day after a night with several dark and moonless hours—a condition known to favor vampire bat attacks (II, 12).

### San Carlos

The data collected 17 days after treatment at San Carlos are shown in Table 6. It was found that only eight cattle showed evidence of recent bites, a reduction of 75.5 per cent. It is also important to recall

<table>
<thead>
<tr>
<th></th>
<th>San Carlos</th>
<th>San Pedro</th>
<th>Kanto</th>
<th>Ojo de Agua</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No. of cattle examined</strong></td>
<td>40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>49&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>No. of cattle bitten</strong></td>
<td>38</td>
<td>8</td>
<td>25</td>
<td>4</td>
</tr>
<tr>
<td><strong>No. of vampire bites</strong></td>
<td>81</td>
<td>10</td>
<td>52</td>
<td>5</td>
</tr>
<tr>
<td><strong>Avg. No. of bites per bovine</strong></td>
<td>1.7</td>
<td>0.2</td>
<td>2.1</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>% of cattle bitten</strong></td>
<td>67.3</td>
<td>16.3</td>
<td>100</td>
<td>16.0</td>
</tr>
<tr>
<td><strong>% reduction of cattle bitten</strong></td>
<td>—</td>
<td>75.8</td>
<td>—</td>
<td>84.0</td>
</tr>
<tr>
<td><strong>% reduction of bites</strong></td>
<td>—</td>
<td>87.7</td>
<td>—</td>
<td>90.4</td>
</tr>
</tbody>
</table>

<sup>a</sup>There were 49 cattle in the San Carlos herd, but 9 escaped from the chute at the time of the first visit and could not be examined or treated.

<sup>b</sup>All the cattle at the ranch.
that nine animals had previously escaped from the chute at San Carlos, making it impossible to treat them or to determine whether or not they had been bitten.

The total number of recent vampire bites counted at the second evaluation was 10, compared with the 81 counted before treatment—indicating an 87.5 per cent reduction. The average number of bites per animal, originally 1.7, was thus reduced to 0.2.

San Pedro

The reexamination (see Table 6) was carried out 16 days after treatment. Only four recently bitten animals and five bites were observed, indicating respective reductions of 84.0 and 90.4 per cent. The average number of bites per animal fell from 2.1 to 0.2.

Kanto

Reexamination data for Kanto, shown in Table 6, were collected 14 days after treatment. Out of 276 cattle reexamined, recent bites were observed on 21, the total number of recent bites observed being 23. Hence the apparent number of cattle attacked dropped 90.2 per cent, and the apparent incidence of recent bites fell 96.4 per cent. The average number of bites per animal examined was 2.3 before treatment and 0.08 after treatment.

Ojo de Agua

On this ranch reexamination data (Table 6) were collected 14 days after treatment. A total of 12 recent bites were found on nine cattle, indicating respective reductions of 93.2 and 76.3 per cent. The average number of bites per animal dropped from 4.5 to 0.3.

Discussion and Conclusions

If we assume that the most practical technique for controlling a pest is apt to be one allowing large numbers of people to participate without special training, then it follows that systemic treatment of cattle is the most suitable method for controlling vampire bats. This method, which can be applied by the rancher himself, makes it unnecessary to train personnel in bat identification and handling, to engage in the risky work of entering bat roosts, or to employ special equipment such as mist nets, traps, gloves, lanterns, and so forth. Moreover, the vampires' special feeding habits ensure that the method can selectively and effectively control vampire bats without inflicting any harm whatever on beneficial non-vampire bat populations.

The results obtained from our studies indicate that warfarin, when used under controlled laboratory conditions, can produce 100 per cent mortality in vampires exposed one night to treated cattle for four consecutive nights after treatment. In this field, treatment was found to reduce the number of cattle bitten by 75.8 per cent at San Carlos, 76.3 per cent at Ojo de Agua, 84.0 per cent at San Pedro, and 90.1 per cent at Kanto when the animals were reexamined 14 to 17 days after treatment. It should be recalled, however, that these tests were conducted at small ranches with precise territorial boundaries that were obviously not respected by vampire bats. In fact, studies of banded vampires by Villa (13) and Forment, Schmidt, and Greenhall (14) have revealed a considerable change in individual roost populations, even when the size of the vampire colony in the roost studied remained stable. Wimsatt (15), in an earlier work, concluded that vampires in a colony represent part of a large mobile vampire bat community.

Looking only at the number of bites, it is significant that reductions ranging from 87.7 to 96.5 per cent were obtained with
Vampirinip III. Nevertheless, the available information on the bats' mobility suggests it would be advisable to treat cattle within the context of a well-established program that operates in clearly-defined zones that take account of natural barriers and the vampires' established areas of activity, so as to obtain the optimum degree of control.

Regarding the pharmacologic and toxicologic tests, results of the first experiment indicated clearly that leukocyte, erythrocyte, and hemoglobin values were similar for the treated and control cattle, and were within the normal ranges defined by Benjamin (6), Miller (7), and Albritton (8). The physiological indicators (rectal temperature, heart rate, and respiration rate) that were measured in experiment 1, experiment 3 (in pregnant cows and calves), and experiment 4 (using progressively larger doses of anticoagulant) consistently remained the normal ranges cited by Dukes (9).

Lags in prothrombin times (evaluated in experiments 1, 2, and 3) were invariably observed 24 hours after the cattle had been treated. These lags reached a maximum some 72 hours after treatment and then gradually declined, with prothrombin times returning to normal between 7 and 10 days after treatment. These results may be explained by the fact that the warfarin in Vampirinip III acts on the liver, competing with vitamin K in the formation of prothrombin and producing hypothyrombinaemia that delays the coagulation process.

In this regard, Goodman and Gilman (10) have reported that therapeutic doses of warfarin in humans cause a 75 to 85 per cent reduction in prothrombin activity, and that this reduced level of activity can be maintained for over a year by continued drug treatment without ill effect. Also, McGirr and Papworth (16) report that a yearling heifer fed warfarin in doses of 50 mg/kg for 10 days showed no symptoms except reduced coagulation time. In experiment 2, where calves were given milk from treated cows, average prothrombin times remained at their initial levels, indicating that the active principle in Vampirinip III was either not eliminated in milk sufficiently to produce changes in the calves' coagulation times, or else it reached the milk in an inactive form.

Experiment 3, which evaluated changes in the prothrombin times of calves and pregnant cows, showed that of the animals tested only the 40-day-old calves showed any really marked prothrombin time delays. As previously noted, this can be attributed to the fact that cattle at this age normally have a vitamin K deficiency; however, even in such animals the reduced level of prothrombin activity was found to be within a range regarded as adequate for humans receiving anticoagulant therapy. Accordingly, it may be concluded that treatment with Vampirinip III can be recommended for cattle at any stage of physiological development. Where problems may arise, and even this remains to be determined, is in treatment of cows going through the last days of pregnancy, just before calving, when the period of maximum prothrombin time reduction could coincide with delivery. Though it is of course true that under field conditions no rancher treats cows at this time, it should also be pointed out that in experiment 4 the cattle in one group receiving the usual dose of warfarin (5 mg/kg) were dehorned 24 hours after treatment. These cattle experienced no hemorrhage problems, despite the fact that no countermeasures were taken at the time.

In experiment 5, which measured the levels of radioactive warfarin in the tissues and milk of cattle treated with Vampirinip III, the levels of radioactivity found in the various tissues examined were generally very low. Because of this, it was not possible to distinguish between warfarin and its metabolites. Assuming no warfarin to have been metabolized, the highest concentration found in liver samples was 7.12 ppm; this
level does not create any problem for human consumers, since one would have to eat 3.5 kg of this liver in order to ingest a minimum therapeutic dose (25 mg)—therapeutic treatment generally entailing an initial dose of 25-75 mg followed by maintenance doses of 5-10 mg per day. It should also be recalled that these levels of anticoagulant were detected in animals sacrificed 120 hours after treatment, because in practice no rancher would be likely to treat an animal scheduled for slaughter 5 days hence. The highest level of warfarin found in milk (discounting strong evidence of metabolic breakdown) was 0.15 ppm—indicating that people could not ingest a minimum therapeutic dose even by drinking many times the entire amount of milk produced at this milking.

In sum, these experiments strongly suggest that the treatment described poses no danger to human health. Despite this fact, however, we believe it important that further studies designed to provide complete assurance of safety be carried out.

In concluding, we also wish to note some of the special advantages of the method we have described. For one thing, all previous vampiricides were insoluble and so could not be administered parenterally. The only systemic method available was the one developed by Thompson (5), in which diphenadione was suspended in carbapol, an agglutinin, at 0.05 per cent and was injected into the rumens of the cattle. Since the active principal in Vampirinip III will dissolve in solvents innocuous to cattle, it can therefore be administered by routine intramuscular injection—a feature offering considerable practical and economic benefits.

It should be added that warfarin injected intramuscularly, as in this study, has been found to have longer-lasting vampiricidal effects than diphenadiones injected into the rumen. Under practical field conditions the longer period of effectiveness is a great advantage; for it assures that virtually all local vampires will feed at least once during this period, and overcomes the problem posed by occasional rainy or moonlit nights that discourage bat attacks.

Regarding possible toxic effects of diphenadione on cattle and humans, Thompson et al. (5) and Bullard et al. (17) both reported injection of vampiricidal amounts into cattle rumens posed no problems of this kind. It is well to emphasize, however, that these conclusions were based on residue levels found in samples of various cattle tissues examined 30, 60, and 90 days after treatment, and on biological tests employing rats fed on those samples.

Additional information was reported by the Nicaraguan delegation to the First Symposium on Hematophagous Bats and Problems Associated with Paralytic Rabies held at Managua, Nicaragua, on 28-30 June 1976. The delegation reported that problems had been encountered with intoxication of young cattle given intrarumenal injections of diphenadione, and that nine of these animals had died. As a result of this experience, the Nicaraguans stated that they neither practiced nor recommended this treatment for cattle under 1 year of age.

As already noted, our own study of possible toxic effects employed warfarin labeled with carbon 14 to analyze tissue residues. Determination of radioactive residue levels was accomplished with the liquid scintillation method on animals slaughtered 5 and 16 days after treatment. The results, previously cited, do not suggest the presence of any toxicity problems. With regard to prothrombin times, the only animals tested that showed a marked lag after treatment were the 40-day-old calves. These findings have been supplemented by the not yet published results of work by Barrenechea et al. (18) that found adequate prothrombin times in 3-month-old calves injected intramuscularly with warfarin and that detected no clinical symptom of intoxication for a period of 3 months after treatment.
This article describes development and testing of a new method for control of vampire bats. The method involves intramuscular injection of cattle subject to vampire attacks with a warfarin-containing preparation named "Vampirinin III."

Laboratory tests of this procedure showed 100 per cent mortality among bats feeding once on appropriately treated cattle for 4 days after treatment. Field tests at four ranches achieved substantial reductions of 87.7, 90.4, 93.2, and 96.5 per cent in the incidence of vampire bites.

Pharmacologic and toxicologic tests indicated that the rectal temperatures, heart rates, and respiration rates of treated cattle invariably remained within normal ranges. Hematic testing also showed that leukocyte, erythrocyte, and hemoglobin levels remained similar for treated and untreated cattle. The only change observed among treated cattle was a lag in prothrombin times, beginning the first day after treatment. This lag reached a maximum level 3 days after treatment and then gradually abated, prothrombin times returning to pre-treatment levels 7 to 10 days after treatment.

Warfarin residue levels in cattle milk and tissues were investigated by injecting cattle with warfarin containing carbon 14. It was found that these residue levels were very low and appeared to pose no public health problem of any kind.

Overall, these test results strongly suggest that the procedure described is both safe and effective, a significant finding in view of the fact that this procedure has important advantages over other available techniques for controlling vampire bats.

**REFERENCES**


The Durable Armadillo and Its Importance to Medical Research

Some general considerations would suggest that immunologically the armadillo is a highly competent animal in its natural environment and in the laboratory environment. Autopsy of several hundred animals at the Pan American Center for Research and Training in Leprosy and Tropical Diseases (CEPIALET) in Caracas, Venezuela, has revealed an extremely low incidence of animals with naturally occurring tumors; ulcers, mycotic infections, and other signs of chronic infection are infrequent. After successful adaptation to laboratory conditions, many animals remain in good health for a number of years. The evolutionary history of this animal, largely unchanged for centuries, suggests its highly successful adaptation, and one must consider immunological competence as one aspect of successful adaptation, even if it were to be achieved by mechanisms of which little is known.

***

The fact that Dasypus sabanicola presents at least two types of responses—systemic lesions similar to those described in D. novemcinctus and lesions which have an epithelioid structure, without acid-fast bacilli—enhances the interest in this species as a model for the study of leprosy. The potential of D. sabanicola as an experimental animal for laboratory purposes makes all the more urgent the study of its physiological and ecological characteristics, especially its breeding patterns in captivity. Observation of breeding on four different occasions in the CEPIALET laboratory has led to the conclusion that this problem could eventually be solved and that a research project directed to this end would be most desirable.

---