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The effect of Imidocarb treatment on Babesia in the bovine and the tick (Boophilus microplus)

3. AUTHOR(S)
Kuttler, K.L.; Graham, O.H.; and Trevino, J.L.

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The effect of Imidocarb treatment on *Babesia* in the bovine and the tick (*Boophilus microplus*)*

K. L. KUTTLER

Institute of Tropical Veterinary Medicine, Texas A & M University,
College Station, Texas 77843 USA

O. H. GRAHAM AND J. L. TREVINO

United States Livestock Insects Laboratory ARS, USDA, Kerrville, Texas 78028 USA

Treatment of calves with 5 mg/kg Imidocarb (3,3'-bis(2-imidazolin-2-yl)carbanilide dipropionate) given intramuscularly 14 days before and 14 days after exposure to *Babesia* infected *Boophilus microplus* larvae rendered the next generation of larvae incapable of transmitting *Babesia* infection. When administered to calves 14 or 28 days before tick exposure, the drug prevented the development of clinical babesiosis; the larval progeny of ticks reared on the calf which was treated 28 days before infestation were infective. Treatment of a calf 42 days before exposure to infective larvae did not prevent the development of a *Babesia* parasitaemia but appeared to reduce the severity of infection.

THE EFFICACY OF Imidocarb† (3,3'-bis(2-imidazolin-2-yl)carbanilide as dipropionate or dihydrochloride) in the treatment and prophylaxis of bovine babesiosis has been reported. Beveridge (1969) found Imidocarb more effective than quimurone, diamidine, and amicarbalide derivatives in preventing *Babesia rodhaini* infections in mice and rats. This compound was recognised as an active and effective drug for the treatment of *B. rodhaini* by Schmidt *et al* (1969). Callow and McGregor (1970) observed that a dose of 2.0 mg/kg Imidocarb was adequate to eliminate *Babesia argentina* infections in cattle, and that 0.6 mg/kg was sufficient to eliminate a *Babesia bigemina* infection in splenectomised steers. In a similar study, Brown and Berger (1970) reported that 1 mg/kg injected intramuscularly (im) eliminated *B. bigemina* infections in cattle.

Callow and McGregor (1970), Hart *et al* (1971) and Roy-Smith (1971) observed a prophylactic effect against *Babesia*. Working with mixed *Babesia* infections (*B. argentina* and *B. bigemina*), Todorovic

et al (1973) reported that 2 mg/kg Imidocarb prevented infection for 20 days, when the challenge consisted of *Babesia* infected blood, and for up to 15 weeks under field challenge.

This study was made to measure the duration of drug induced resistance in calves when exposed to *Babesia* spp via an experimental tick challenge and the influence of this treatment on the subsequent infectivity of the ticks.

Materials and methods

Eight dairy type, bull calves approximately four to six months old were used. Calves 1, 2, 3 and 4 were treated with 5 mg/kg Imidocarb dipropionate injected im. Calf 1 was treated 14 days after the release of *Babesia* infected larvae, at a time when *Babesia* parasitaemia was present. Calves 2, 3 and 4 were treated 14, 28 and 42 days before larval release. Calves 5, 6, 7 and 8 remained as untreated controls, paired with calves 1, 2, 3 and 4, respectively.

Infected *Boophilus microplus* larvae were obtained from a single pool of eggs from several engorged females that had fed on a *Babesia*-infected calf. From this pool the eggs were divided into 500 mg aliquots and allowed to hatch into larvae. Each of the eight calves were then in turn infested by 500 mg larvae from this common pool. The *Babesia* infections observed resembled *B. argentina* morphologically, but the possibility exists that *B. bigemina* was also present.

Following infestation with *B. microplus* larvae, calf temperatures were taken daily. Blood samples in EDTA were taken from all experimental calves at weekly intervals until evidence of a febrile response occurred, and then were taken daily. Giemsa-stained thin blood smears were prepared and examined for evidence of haemoparasites. A packed red cell volume (PCV) was determined on each sample, using a micro-haematocrit centrifuge.

A total of 20 engorged female ticks were collected

* Mention of a proprietary product in this paper does not constitute an endorsement of this product by the USDA.
† Burroughs Wellcome and Company, Inc., Raleigh, North Carolina.

from each calf. Each of the engorged females, at the time of collection, and the resulting egg mass 18 days later, were weighed. The egg/female weight ratio was determined to evaluate further possible influence of treatment on the female tick and her fecundity. After weighing each egg mass originating from a single calf, they were pooled and divided into 500 mg aliquots for future use.

When ready to feed, the larval progeny of engorged female ticks collected from calves 1 and 5, 2 and 6, and 3 and 7 were released on each of six susceptible splenectomised calves to determine larval infectivity. The diagnosis of *Babesia* infection and parasitaemia in calf 4 was confirmed by inoculating a splenectomised calf with 10 ml of its blood intravenously, collected 42 days after the fever response.

Results

The influence of Imidocarb treatment on *Babesia*

infection in calves and on *Boophilus* ticks is tabulated in Tables 1 and 2, respectively.

Calf 1 developed signs of *Babesia* infection before treatment on day 14. Calf 1 recovered following treatment. The larval progeny of ticks recovered from calf 1 were not infective for *Babesia* when placed on a susceptible splenectomised calf.

Calf 2, treated 14 days before tick infestation, failed to develop evidence of *Babesia* infection. Larval progeny of ticks recovered from calf 2 were not infective for *Babesia* when placed on a susceptible splenectomised calf.

Calf 3, treated 28 days before tick infestation, failed to develop evidence of *Babesia* infection. The larval progeny of ticks recovered from calf 3 transmitted *Babesia* infection to a splenectomised calf.

Calf 4, treated 42 days prior to tick release, developed mild clinical signs of babesiosis. The occur-

TABLE 1: Prophylactic effect of Imidocarb against *Babesia* infection in calves and ticks

Calf number	Day of treatment*	Response to tick infestation			<i>Babesia</i> parasitaemia	Infectivity of larval progeny
		First day of fever	Min PCV	Max temp (°C)		
1	+14	13	10	40.3	Pos.	Neg.
2	-14	None	25	39.1	Neg.	Neg.
3	-28	None	26	38.9	Neg.	Pos.
4	-42	14	30	40.4	Pos.†	NT
5	None	13	12	40.7	Pos.	Pos.
6	None	13	13	41.1	Neg.‡	Pos.
7	None	13	16	40.2	Pos.	Pos.
8	None	15	25	40.4	NT	NT

* Calf 1 was treated 14 days after tick exposure; calves 2, 3 and 4 were treated 14, 28 and 42 days before tick exposure.

† Confirmed by sub-inoculation.

‡ Negative on thin smear, but clinical signs of babesiosis were observed.

NT = No test.

TABLE 2: Influence of Imidocarb treatment on size and fecundity of *B. microplus* ticks

Calf number	Avg wts of 20 engorged females (mg)	Avg egg wts from 20 engorged females (mg)	Ratio of egg engorged female weights
1	404 ± 32	228 ± 28	0.56 ± 0.06
5	409 ± 36	235 ± 29	0.57 ± 0.05
2	348 ± 34	203 ± 21	0.58 ± 0.04
6	438 ± 58	248 ± 54	0.56 ± 0.12
3	442 ± 41	263 ± 26	0.59 ± 0.04
7	385 ± 42	128 ± 30	0.34 ± 0.09
4	509 ± 43	304 ± 60	0.60 ± 0.12
8	461 ± 40	253 ± 40	0.55 ± 0.09

±: Standard deviation.

rence of infection in calf 4 was confirmed by inoculating 10 ml of whole blood into a susceptible splenectomised calf, which died of acute babesiosis (*B. argentina*) 14 days after injection.

Control calves 5, 6, 7 and 8, receiving no treatment, developed evidence of babesiosis after infestation with *B. microplus* larvae. The larval progeny from calves 5, 6 and 7, when released on splenectomised calves, consistently produced babesiosis.

The average weights of 20 engorged female ticks collected from all calves are recorded in Table 2. The average egg yield from each female plus the ratio egg weight/female weight are also recorded. No clear pattern of influence attributable to either treatment or infection on female weights or fecundity was evident.

Discussion

The apparent success of Imidocarb as a therapeutic and chemoprophylactic agent against bovine babesiosis in both the vertebrate and invertebrate hosts suggests the possible use of this compound in future control programmes. In situations where tick eradication is not feasible, or is impractical, *Babesia* control and possible eradication might be considered using this compound. Additional field studies would be required and the practical dose range and treatment interval determined, but if *Babesia* can be eliminated in both hosts then theoretically with repeated treatments the reservoir of infection could be eliminated even in the presence of *Boophilus* ticks.

In these trials it is not clear whether the drug killed the *Babesia* spp in the tick or if the drug merely prevented the development of the organism in the vertebrate host and hence interfered with reinfection of the tick. It was, regrettably, not determined if calf 3 actually developed a sub-clinical infection, hence reinfected ticks, or if the ticks retained infection to the next generation, in the absence of infection in calf 3.

Differences in female weights and egg/female weight ratios occurred but, in view of the large number of variables and the limited observations,

it is difficult to ascribe these differences to either the drug or infection. The drug did not have any lethal effects on the ticks.

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