Journal of the American Mosquito Control Association, 10(1):70–73, 1994

EFFECTS OF IVERMECTIN ON SURVIVAL, FECUNDITY, AND EGG FERTILITY IN *CULICOIDES VARIIPENNIS* (DIPTERA: CERATOPOGONIDAE)[1]

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ABSTRACT. Colonized female *Culicoides variipennis* were fed through an artificial membrane on sheep blood meals containing 0–1.0 μg/ml of ivermectin. Significant mortalities were present at 48 h postfeeding, with probit analysis yielding an LC_{50} of 0.35 μg/ml and a slope of 4.12. All dosages caused significant decreases ($P < 0.01$) in egg production and sporadic decreases in egg hatch, with no eggs produced at 1.0 μg/ml. There was a negative linear correlation ($r^2 = 0.91$) between dose rate and number of eggs produced per living female. An inoculation of ivermectin at the recommended dosage of 200 μg/kg of body weight would not produce the serum concentration that could be expected to have noticeable effects on bloodfeeding *C. variipennis*.

INTRODUCTION

Bluetongue (BLU) disease of ruminants is widespread in the USA (Metcalf et al. 1981), with the biting midge *Culicoides variipennis* (Coq.) the principal vector of the BLU viruses (Price and Hardy 1954). Recommendations for the control of this disease in North America have been directed primarily against the vector, with emphasis on water and waste management and the use of insecticides (Holbrook 1988, Mullens and Rodriguez 1990).

Certain oleandrose disaccharide derivatives of pentacyclic lactones (avermectins) isolated from the soil microorganism *Streptomyces avermitilis* (Burg et al. 1979) have demonstrated a broad spectrum of activity against parasitic infections in vertebrates (Campbell 1989). Ivermectin also has been shown to affect hematophagous arthropods. At higher dosages, effects have been paralysis and death, reduced adult survival, and reduced survival of larvae of the subsequent generation; lower dosages have produced blood digestion without ovarian development, and decreased fecundity and egg hatch (Focks et al. 1991, Mahmood et al. 1991). In Australia in tests against *Culicoides brevitarsis* Kieffer, ivermectin administered subcutaneously to cattle at a rate of 200 μg/kg gave a 99% mean control for 10 days posttreatment and 47% mortality at 24 days (Standfast et al. 1984).

Because of its broad spectrum of activity and its demonstrated effects on at least one species of *Culicoides*, ivermectin appeared to be a promising control option for the management of vector-borne bluetongue in domestic ruminants in the USA. We report here effects of ivermectin in blood on the mortality, fecundity, and egg viability of *C. variipennis*.

MATERIALS AND METHODS

The ivermectin used in this study was formulated as a technical grade powder, provided by Merck and Co., Inc., Rahway, NJ. It was diluted in dimethylsulfoxide to prepare a stock solution containing 100 μg/ml, with appropriate volumes diluted in 10 ml of defibrinated sheep blood to give concentrations of 0.0, 0.2, 0.4, 0.6, 0.8, and 1.0 μg/ml, a range indicated by preliminary trials. Preliminary studies demonstrated that this amount (0.1%) of dimethylsulfoxide alone in blood had no effects on mortality, fecundity, or egg hatch. These doses and a blood-only control were placed in artificial feeders equipped to warm and mix the blood. The insects used in this study were from a colony of *C. variipennis* that was established in 1957 from insects collected near Sonora, TX (Jones 1960). Females (48–72 h postemergence) were allowed to feed through artificial silicone membranes (Hunt and McKinnon 1990) over a 3–4-h period in subdued lighting at room temperature. The insects were anesthetized with a 15-sec exposure to CO_2 gas, then sorted on a chill table. Fifty fully engorged females and 10–15 males were placed in each of 2 cages/treatment. The cages, 0.24-liter ice cream cartons covered with fine-mesh organdy, were provided with oviposition containers that held pads of moist cotton covered with filter paper. Each cage had a vial containing a 10% sucrose solution and a cotton dental wick. The insects were held at 26 ± 1°C, 50–60% RH, and a 13L: 11D photoperiod, standard for colony production.

[1] This paper reports the results of research only. Mention of a commercial or proprietary product does not constitute a recommendation or an endorsement of this product by the U.S. Department of Agriculture.

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Table 1. *Culicoides variipennis*: survival, eggs produced/living female, and percent egg hatch following feeding to repletion through an artificial membrane by 100 nulliparous females on defibrinated sheep blood containing doses of 0–1.0 μg/ml ivermectin.

Ivermectin dose (μg/ml)	Parameter	Days posttreatment						
		1[1]	2[1]	3	4	5	6	7
Control	% survival	99	96	85	81	75	75	61
	Eggs/live ♀			30.3	44.8	9.6	0	2.7
	% hatch			95.0	93.7	98.1	—	74.6
0.2	% survival	85	78	58	51	48	30	28
	Eggs/live ♀			20.3	41.4	9.6	0.9	3.3
	% hatch			90.9	73.4	90.7	100	82.8
0.4	% survival	94	44	18	15	13	13	13
	Eggs/live ♀			18.0	33.8	0	0	1.0
	% hatch			83.7	72.9	—	—	46.2
0.6	% survival	85	14	9	8	7	7	0
	Eggs/live ♀			0	8.0	0	0	0
	% hatch			—	0	—	—	—
0.8	% survival	85	7	1	0	0	0	0
	Eggs/live ♀			13.4	0	—	—	—
	% hatch			76.6	—	—	—	—
1.0	% survival	77	2	0	0	0	0	0
	Eggs/live ♀			0	—	—	—	—
	% hatch			—	—	—	—	—

[1] Oogenesis, no eggs laid.

Survival counts were made daily for 7 days postfeeding, with dead insects left in the cages. Beginning at day 3, when oviposition commenced, the oviposition containers were changed daily when eggs were present. The egg pads were kept moist in covered petri dishes at 26 ± 1°C until hatching was complete (approximately 2 days). Total eggs and number hatched were recorded. The daily production of eggs/living female was calculated as the number of eggs collected divided by the number of living females present at the start of that egg-laying period. The number of eggs per living female per day for the entire test period was calculated as the total number of eggs produced divided by the total number of living female egg-laying days during the egg production period. (For example, in the controls, there were 7,797 eggs produced from days 3 to 7. The cumulative female egg laying days were 96 [day 2] + 85 [day 3] + 81 [day 4] + 75 [day 5] + 75 [day 6] = 412.)

A contingency chi-square analysis indicated no significant cage effects, so the 48-h mortality data from the 2 cages at each ivermectin dosage were combined and corrected for control mortality. An LC_{50} was estimated by probit analysis (Finney 1971) using a computer program (Daum 1970) customized at this laboratory. The data on egg production/living female/day and fertility were subjected to contingency chi-square and regression analyses using SYSTAT software (Wilkinson 1989).

RESULTS

The survival of *C. variipennis* females for 7 days postengorgement on blood meals with or without ivermectin, eggs produced/living female/day, and daily hatch rates are shown in Table 1. At day 1, mortalities of 23% at the highest ivermectin treatment (1.0 μg/ml of blood), 6–15% in the 0.2–0.8 μg/ml treatments, and 1% in the controls, were not significantly different. At day 2, probit analysis yielded a slope of 4.21 (SE = 0.39), with an LC_{50} of 0.32 μg/ml (SE = 0.02; F = 83.67, df 1,3). The 100% mortality level was reached at the 1.0 and 0.8 μg/ml dosages on days 3 and 4, respectively, and on day 7 at the 0.6 μg/ml dosage.

Eggs were deposited during days 3–7, with eggs produced by females at all dosages below 1.0 μg/ml. However, there were such high mortality rates during the egg-laying period at doses above 0.2 μg/ml that the numbers of eggs were insufficient for statistical analyses of daily production. A Pearson chi-square analysis conducted on the data for eggs/living female at the 0 and 0.2 μg/ml doses from days 3 to 7 showed no significant differences. There was a significant negative linear relationship ($r^2 = 0.91$; $y = 0.88 - 9.54^{-3x}$)

between dose (the independent variable) rate and total number of eggs produced/living female/day (Table 1). Egg hatch in general was not affected markedly, though there was significantly reduced egg hatch at doses >0.4 μg/ml (Table 1).

DISCUSSION

Following a subcutaneous injection in cattle of 200 μg of ivermectin/kg of body weight, peak plasma concentration of 0.044 μg/ml is reached at one day postinoculation (Fink and Porras 1989). We estimate from Standfast et al. (1984) the LT_{50} (lethal time for 50% mortality in the test population) of *C. brevitarsis* at the 200 μg dose rate to be 17 days postinoculation. By extrapolation of the data from Fink and Porras (1989), the plasma concentration of ivermectin at 17 days would be *ca.* 0.003 μg/ml. This suggests that the effective dose for *C. brevitarsis* is *ca.* 105 times less than that for *C. variipennis* (0.003 μg/ml vs. 0.35 μg/ml). It is therefore unlikely that blood levels of ivermectin in cattle (and possibly sheep) would affect the survival of *C. variipennis* fed on an animal treated at the recommended rate of 200 μg/kg of body weight.

The slower rate of egg development reported in female *Aedes aegypti* (Linn.) treated with ivermectin (Mahmood et al. 1991) was not evident in *C. variipennis,* because both untreated flies and those treated with 0.2, 0.4, or 0.8 μg/ml began producing eggs on day 3. Nevertheless, at dose rates of 0.2–1.0 μg/ml of blood, the total number of eggs, the number of eggs/living female produced, and the egg hatch rate decreased significantly as the rate of ivermectin increased. It appears that 200 μg of ivermectin/kg of body weight would not have a meaningful effect on the reproductive capacity of *C. variipennis,* because the highest plasma concentration (0.044 μg/ml at 24 h postinoculation) is still 4.5 times less than the lowest concentration of ivermectin (0.2 μg/ml) used in these tests.

Neville (1978) proposed placing cattle, a preferred host for *Culicoides imicola* Kieffer, with sheep to differentially attract host-seeking females and thus reduce the attack rates on the sheep. Treating those cattle with very high rates of ivermectin might significantly reduce the vector population. Dosages up to 6,000 μg/kg of body weight (30 times the recommended rate) produce an estimated peak blood concentration of 1.3 μg/ml and have no obvious ill effects in cattle (Pulliam and Preston 1989). With a biological half-life of 3 days (Fink and Porras 1989), the concentration of ivermectin could be high enough for a few days to significantly affect mortality, egg production, and egg fertility in *C. variipennis sonorensis* females.

The insects used in this evaluation represent only one of the 5 subspecies of the *C. variipennis* complex (Wirth and Jones 1957) present in the USA and *C. variipennis sonorensis* Wirth and Jones were from a colony maintained continuously for more than 30 years. There could be differences in natural populations of *C. variipennis sonorensis,* as well as between the other subspecies, *C. variipennis albertensis* Wirth and Jones, *C. variipennis australis* Wirth and Jones, *C. variipennis occidentalis* Wirth and Jones, and *C. variipennis variipennis* Wirth and Jones, and between populations within those subspecies. Also, other formulations of ivermectin, including a pour-on that has shown topical activity against a number of ectoparasites of cattle, may prove effective for the control of adult *Culicoides.*

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