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SURVIVAL, FECUNDITY, AND EGG FERTILITY OF *CULICOIDES VARIIPENNIS* (DIPTERA: CERATOPOGONIDAE) FED ON CALVES INOCULATED WITH IVERMECTIN[1]

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ABSTRACT. Female *Culicoides variipennis* were fed at 1–18 days postinoculation on calves treated with a commercial injectable formulation of ivermectin at the recommended rate of 200 μg/kg of body weight. There were no significant differences between treated and untreated animals in the survival rate of the flies at 24 h and 48 h postinoculation and the number of eggs produced per living female. There was a significantly greater mean hatch rate of eggs from flies fed on the untreated animals (65.8%) than those fed on the treated animals (52.8%). The recommended use rate of ivermectin does not appear to provide significant mortality for female *C. variipennis*, nor affect egg deposition or hatch rate sufficiently to reduce vector populations.

INTRODUCTION

Culicoides variipennis (Coq.) is the principal vector of bluetongue viruses (Price and Hardy 1954) and epizootic hemorrhagic disease viruses (Jones et al. 1977) of ruminants in North America. *Culicoides variipennis* has been shown also to be a competent vector of exotic African horsesickness viruses (Boorman et al. 1975) and of indigenous *Onchocerca cervicalis* Railliet and Henry (Jones and Collins 1981). Control recommendations for *C. variipennis* have emphasized livestock production practices, water and waste management, and the use of larvicides and adulticides (Jones et al. 1981; Holbrook 1985, 1988; Mullens and Rodriguez 1990).

Ivermectin, a macrocyclic lactone glycoside isolated from the soil microorganism, *Streptomyces avermitilis* (Chen et al. 1989), has demonstrated a broad spectrum of activity against parasitic infections in vertebrates (Campbell 1989), and has been evaluated against a variety of hematophagous insects. The recommended dose of 200 μg/kg of body weight, to be administered subcutaneously in the shoulder, was selected after numerous dose–titration trials (Benz et al. 1989). In studies with cattle in Australia, a single subcutaneous inoculation of ivermectin at 200 μg/kg of body weight in 4 cattle gave mean mortalities at 48 h postfeeding of >99% for 10 days and >40% for 18 days to *Culicoides brevitarsis* Kieffer (Standfast et al. 1984). The following studies were conducted to assess the effects of a single dose of ivermectin at 200 μg/kg of body weight in calves against *C. variipennis*.

MATERIALS AND METHODS

Four mixed-breed beef calves from the current year's crop that were 6 months of age at the beginning of the tests were utilized. They were housed indoors in separate rooms for several weeks prior to testing to decrease possible contact with biting insects and to facilitate handling. The same individual calves were used as control and treatment animals (2 each) in 2 tests conducted at intervals of 93 days, more than 3 times the period when invermectin is detectable in blood plasma in cattle (Fink and Porras 1989).

The invermectin used was a commercially available aqueous formulation containing 1% w/v ivermectin (Ivomec, Merck and Co., Inc., Rahway, NJ 07065). It was inoculated subcutaneously into the neck of a calf at a single dosage of 200 μg/kg of body weight.

The *C. variipennis* adult females used for these tests were from a colony maintained continuously at the Arthropod-borne Animal Diseases Research Laboratory since 1957, and originated from a collection in Sonora, TX, USA (Jones 1960). In pesticide trials, individuals from this colony have produced results similar to those from wild populations (Holbrook 1982). Two hundred and fifty pupae of the same age were placed in emergence cages and held in an incubator at 26 ± 1°C with a 13L:11D photoperiod. The adults used were those that emerged on days 3 to 4. The flies were held until day 5 with water provided in a vial with a dental cotton wick. These 24–48-h-old flies were anesthetized for 15 sec with CO_2 gas and transferred to 4 feeding cages, 0.24-liter ice cream containers covered with a tightly stretched nylon stocking. A cage was placed against a shaven area on an animal and held with an elastic bandage for a feeding period of 30 min. After feeding, the cages were placed on a chill table and all fully engorged females

[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.

Table 1. Survival, fecundity, and fertility in fully engorged *Culicoides variipennis* females fed on untreated calves and calves treated with ivermectin at 200 μg/kg of body weight.

		Test 1				Test 2			
		% survival		No. fe-	Eggs/female	% survival		No. fe-	Eggs/female
Day	Treatment	24 h	48 h	males	(% hatch)	24 h	48 h	males	(% hatch)
1	Control	95.2	88.9	56	55.9 (70.8)	65.5	46.6	27	63.1 (69.5)
	Ivermectin	90.7	66.3	57	58.0 (64.3)	90.1	74.3	75	40.3 (41.7)
4	Control	98.7	97.4	225	47.9 (88.5)	98.6	95.8	68	34.0 (54.6)
5[1]	Ivermectin	96.1	90.3	186	90.4 (48.2)	100	100	32	34.8 (49.7)
8	Control	81.8	61.3	84	83.2 (63.1)	100	98.8	79	36.5 (54.3)
	Ivermectin	92.9	82.6	128	77.6 (50.4)	100	100	53	17.9 (56.9)
11	Control	96.9	93.3	181	84.3 (71.2)	94.8	87.5	84	50.4 (54.9)
	Ivermectin	98.0	96.4	189	127.7 (64.8)	100	100	50	22.2 (43.9)
15	Control	95.7	89.0	146	95.2 (77.4)	98.5	97.7	127	18.1 (59.5)
	Ivermectin	94.9	89.9	160	78.4 (50.8)	96.5	89.6	129	37.0 (42.1)
18	Control	92.4	77.9	113	85.2 (79.4)	96.0	88.0	22	4.9 (46.7)
	Ivermectin	96.6	92.7	216	94.4 (89.9)	96.6	89.8	53	19.3 (30.6)

[1] Test 1, day 5; Test 2, day 4.

and 10–15 males were put in holding cages covered with fine mesh organdy. Flies were supplied a vial of 10% sugar water and held in an incubator at 26 ± 1°C with a 13L:11D photoperiod. A plastic container with a moist cotton pad covered with a disk of filter paper was provided for oviposition. Mortality counts of females were made at 24 and 48 h postfeeding (days 6 and 7). Eggs were laid on moist filter paper disks and collected on days 8, 9, and 10. The "egging" papers were placed in Petri dishes over moisture for 5 days, when counts were made of total hatched and unhatched eggs.

Data on survival, egg production, and egg hatch rate were analyzed by analyses of variance and Scheffe's test using SYSTAT software (Wilkinson 1989).

RESULTS

The data on survival, eggs per living female, and egg hatch for the 2 tests are shown in Table 1. There were no significant differences in survival rates of females at either 24 h or 48 h postfeeding between the untreated animals and the treated animals in either test. However, there were significant differences between mean survival rates of female *C. variipennis* between 24 h ($\bar{x}$ = 94.5%) and 48 h ($\bar{x}$ = 87.2%) postfeeding in both tests ($F = 5.35$; df = 1,46; $P < 0.05$).

There were no significant differences in the numbers of eggs produced by those females fed on the untreated animals or on the treated animals. There were significantly more eggs produced per female in Test 1 than in Test 2 ($F = 42.45$; df = 1,22; $P < 0.0001$). There was a significantly greater hatch rate of eggs from females fed on the untreated animals ($\bar{x}$ = 65.8%) than from those on the treated animals ($\bar{x}$ = 52.8%) ($F = 5.34$; df = 1,22; $P < 0.05$).

DISCUSSION

The same dose rate of 200 μg/kg of body weight that produced 99% mortality for 10 days in *C. brevitarsis* in Australia (Standfast et al. 1984) did not produce significant mortality in *C. variipennis* females in the tests reported here. This dose of 200 μg/kg of body weight produces a peak blood concentration of 44 ng/ml (Fink and Porras 1989). The LC_{50} for *C. variipennis* (Holbrook and Mullens 1994) is 350 ng/ml of blood, and the initial dose would have to be increased to *ca.* 1,600 μg/kg of body weight to produce this blood level. Dose rates of 6,000 μg/kg (30 times the recommended use level) have been administered to cattle without noticeable ill effects (Pulliam and Preston 1989).

The nonsignificant effects of the 200 μg/kg dosage on total egg production and eggs per living female found here are not surprising, as similar results were reported in tests in which the highest blood level of invermectin (1,000 ng/ml) (Holbrook and Mullens 1994) was *ca.* 23 times the estimated blood level in the tests reported here.

The decrease in mean egg hatch rate from the females fed on the treated animals in the current trials was similar to those reported at a 5 times higher concentration of 200 ng/ml of blood (Holbrook and Mullens 1994). Those results were

obtained *in vitro* using defibrinated blood fed to the insects in a membrane feeding system. There may be increased biological availability and enhanced efficacy of ivermectin with an *in vivo* treatment.

The difference in number of eggs produced per female between Test 1 and Test 2 may be linked to increasing sensitivity of the calves to the bites of *C. variipennis* females. Akey et al. (1989) showed that sequential feeding on a naive calf by caged *C. variipennis* eventually produced localized edema, accompanied by a significant increase in the percentage of females with a partial blood meal and a higher than normal percentage of plasma. No local reactions were observed at the site of feeding during Test 1. On day 7 of Test 2, serum was observed transuding from the skin of one control calf at the site where the cage had been fastened, and similar transudates were noted from all 4 calves by day 11. Even prior to the time that these transudates became noticeable, an increased plasma percentage in the blood meal could have affected egg production.

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