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EFFECTS OF IVERMECTIN IN CANINE BLOOD ON *ANOPHELES QUADRIMACULATUS, AEDES ALBOPICTUS* AND *CULEX SALINARIUS*[1,2]

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ABSTRACT. Blood from ivermectin-treated dogs was tested against adult mosquitoes. Mosquitoes were allowed to bloodfeed on mixed breed dogs 4 h after dogs were given oral dosages of ivermectin. In test 1, *Anopheles quadrimaculatus* and *Aedes albopictus* fed on dogs that had been given ivermectin dosages of 0, 6, 12, and 24 μg/kg. In test 2, *Culex salinarius* and *Ae. albopictus* fed on dogs that had been given dosages of 0, 15, 30, 60, and 120 μg/kg. In both tests, mosquitoes were observed for mortality at 1, 12, and 24 h postfeeding. Surviving mosquitoes were observed for oviposition and egg hatching. In the first test, there was a significant increase in mortality and a significant decrease in number of eggs/female and egg hatchability in *An. quadrimaculatus* but not in *Ae. albopictus* ($P < 0.05$). An LD_{50} of 9.9 μg/kg (95% FL 6.0–13.8) was determined for *An. quadrimaculatus*. In the 2nd test, there were no significant differences in any variable for *Cx. salinarius* or *Ae. albopictus*, except that eggs from *Ae. albopictus* had reduced hatching at all dosages.

INTRODUCTION

Ivermectin is a widely used livestock and canine anthelmintic. Many studies have focused on the effect of ivermectins on permanent parasitic arthropods and helminths, but relatively few studies have reported the effects on mosquito biology.

Aedes aegypti (Linn.) fed on rabbits injected with 10 mg/kg of ivermectin showed reduced survival and egg production when compared to females fed on control rabbits (Focks et al. 1991). An LD_{50} of 82 mg/kg was determined for *Culex quinquefasciatus* Say fed on ivermectin-treated mice. *Anopheles stephensi* Liston exhibited 100% mortality after feeding on mice treated with 2.8 mg/kg of ivermectin (Pampiglione et al. 1985). Tesh and Guzman (1990) reported that ivermectin levels of 3.4 and 4.3 ng/ml in human blood caused 50% of *Ae. aegypti* and *Aedes albopictus* (Skuse) eggs to be infertile. They also determined LD_{50}s for *Ae. aegypti* (126 ng/ml), *Ae. albopictus* (208 ng/ml), and *Cx. quinquefasciatus* (698 ng/ml) when they fed on ivermectin-treated human blood. A study of *Anopheles quadrimaculatus* Say showed greater than 90% mortality at 10, 500, and 1,000 μg/kg and 100% mortality at 2,000 μg/kg of ivermectin when mosquitoes fed on treated dogs (Jones et al. 1992).

The objective of this study was to further examine the effects of ivermectin in canine blood on mosquito survivorship, oviposition, and egg viability.

MATERIALS AND METHODS

For test 1, *Anopheles quadrimaculatus* adults were collected July 26, 1992, from resting stations at the University of Arkansas Rice Research and Extension Center (RREC), approximately 13 km east of Stuttgart, AR (Arkansas County). Test mosquitoes were separated by placing collection containers in a chill cabinet (−10°C) for 30 sec and subsequently placing them on a chill table. Unengorged females were transferred by mouth aspirator from the chill table to 24 236-ml cardboard containers (Fonda Group Inc., Union, NJ), with 20 females per container. Each container was covered with nylon mesh screening. The mosquitoes were placed in an insulated chest and transported to Baton Rouge, LA. Adults of *Ae. albopictus* were obtained from a colony at the Calcasieu Parish Mosquito Control District in Lake Charles, LA. They were handled in the manner described previously, and 10 unengorged females were transferred to each of 24 cardboard containers. Additional mosquitoes were available to serve as replacements in the event of enroute mortality.

Twelve mixed breed dogs, located at the Louisiana State University School of Veterinary Medicine in Baton Rouge, were divided into 4 groups of 3 dogs each. Five-milliliter blood samples were taken from each dog immediately prior to treatment. Ivermectin[6] (Merck and Co., Rah-

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[2] Research was conducted in compliance to principles stated in the *Guide for the Care and Use of Laboratory Animals.*

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[6] Mention of a product does not imply a recommendation for use or endorsement for sale by the University of Arkansas or Louisiana State University.

Table 1. Mortality, fecundity and hatch of *Anopheles quadrimaculatus* and *Aedes albopictus* fed on dogs treated orally with ivermectin.[1]

Dose (μg/kg)	*An. quadrimaculatus* Adult 24-h % mortality	*An. quadrimaculatus* Mean no. eggs/ female that laid	*An. quadrimaculatus* % hatch	*Ae. albopictus* Adult 24-h % mortality	*Ae. albopictus* Mean no. eggs/ female that laid	*Ae. albopictus* % hatch[2]
0	3.9a	163.5a	78.8a	2.5a	67.0a	—
6	33.2b	111.3b	80.9a	1.6a	59.3a	—
12	66.9c	111.3b	48.4b	5.0a	59.4a	—
24	65.3c	19.0c	0.0c	1.7a	70.7a	—

[1] Means in the same column followed by the same letter are not significantly different by Duncan's multiple range test ($P < 0.05$).
[2] No data due to technique error.

way, NJ) was diluted with ethyl glycol, a solvent, and a 5-ml volume was administered orally to each dog. One group received the prescribed prophylactic dose of 6 μg/kg, another received 12 μg/kg, and the remaining treatment group received 24 μg/kg. The untreated control dogs were given 5 ml of ethyl glycol. Four hours posttreatment, the time at which blood concentration levels of ivermectin peak (Campbell 1989), the dogs were immobilized with 17.6 mg/kg of thiamylal sodium. Blood samples of 5 ml were taken again, and 81-cm² areas at the shoulder, side, hip, and inside hind leg of each dog were shorn. Two containers of 20 *An. quadrimaculatus* and 2 containers of 10 *Ae. albopictus* were placed on each dog with the mesh screening in contact with the shorn areas. Mosquitoes were allowed to feed for 20 min. Afterwards, each mosquito cage was removed, placed in an insulated chest, and the mosquitoes were provided with a cotton ball soaked in 10% sugar water. A 5-ml blood sample was taken from each dog immediately after mosquito feeding and was subsequently delivered to the diagnostic laboratory of the School of Veterinary Medicine for ivermectin concentration analysis by high-pressure liquid chromatography.

The mosquitoes were transported to the Medical–Veterinary Entomology Laboratory of the University of Arkansas at Fayetteville within 12 h following the conclusion of mosquito feeding. Mortality readings were taken at 1, 12, and 24 h postfeeding. Each mosquito was transferred via aspiration into a 140-ml plastic container (Napco Inc., Fayetteville, AR) containing filter paper and approximately 15 ml of water; the container was then covered with nylon mesh screening. Mosquitoes were observed daily for mortality or oviposition. Deposited eggs were counted and subjected to a hatching solution following incubation following procedures in Gerberg (1970).

The test was repeated (test 2) August 18, 1992, using *Culex salinarius* Coq. and *Ae. albopictus*. *Culex salinarius* were collected from CDC miniature light traps in the vicinity of Baton Rouge. Newly emerged *Ae. albopictus* were obtained from laboratory colonies maintained at the Calcasieu Parish Mosquito Control District and the Orleans Parish Mosquito Control Board in New Orleans. They were chilled and transferred as in the previous test with 20 females/cup for both species.

Two additional dogs were used for a total of 14 dogs. The dogs were divided into 4 groups of 3 and one group of only 2 due to illness in one animal. Blood samples were taken twice before treatment and immediately prior to mosquito feeding. The treatment dosages were increased to 15, 30, 60, and 120 μg/kg, and untreated, with the 30-μg/kg dosage given to the group of 2 dogs. Dosages were administered as in the previous test. The dogs were prepared in the manner described above, and mosquitoes were allowed to feed for 20 min. They were removed and provided sugar water. Mosquitoes were transported to the Mosquito Research Laboratory at the LSU Agricultural Center and observed for mortality at 1, 12, and 24 h postfeeding. They were then transferred to the University of Arkansas laboratory, individually placed into single cups and observed daily for mortality and oviposition.

Following incubation, eggs were stimulated to hatch and recorded as to eclosion success. Data were subjected to analysis of variance and means separated by Duncan's multiple range test. An LD_{50} was determined by probit analysis (SAS Institute 1985).

RESULTS AND DISCUSSION

In test 1, *An. quadrimaculatus* mortality was significantly higher ($P < 0.05$) in the 2 higher levels of ivermectin than the control and lower dosage (Table 1). This agrees with Jones et al. (1992) who found that *An. quadrimaculatus* had increased mortality at ivermectin levels ranging

Table 2. Test 1—average concentration (ng/ml ± SE) of ivermectin in drawn canine blood sampled prior to treatment, 4 h posttreatment and after mosquito feeding.[1]

Dose (μg/kg)	Pre-treatment	4 h post-treatment	After feeding
0	0	0	0
6	0	6 ± 1.0	0.33 ± 0.33
12	0	11 ± 2.0	7 ± 3.6
24	0	16 ± 7.5	19 ± 1.7

[1] Blood samples analyzed by high-pressure liquid chromatography.

from 10 to 2,500 μg/kg. An LD_{50}, 9.9 μg/kg (95% FL 6.0–13.8) was determined for *An. quadrimaculatus* when fed on ivermectin-treated dogs. The average number of eggs laid/female that laid was significantly less in the highest dosage when compared to the control, 19.0 (only one female oviposited) and 163.5, respectively. Hatch data were transformed using arcsine transformation because the range of percent hatch exceeded 40. Untransformed data are presented in the tables. Egg hatchability was significantly greater in the control (78.8%) and lowest dose (80.9%) when compared to hatchability at the 12- (48.4%) and 24-μg/kg dosages (0.0%). The average concentration of ivermectin in the dog blood for each dose is shown in Table 2 and corresponds to mortality differences shown in Table 1.

Aedes albopictus exhibited no significant difference in percentage mortality and average number of eggs/female at these dosages (Table 1). Because the eggs were not hatched no data were available for this variable.

Table 3. Mortality, fecundity and hatch of *Culex salinarius* and *Aedes albopictus* fed on dogs treated with ivermectin.[1]

	Cx. salinarius		*Ae. albopictus*	
Dose (μg/kg)	Adult 24-h % mortality	Mean no. eggs/ female that laid	Adult 24-h % mortality	Mean no. eggs/ female that laid
0	7.5a	45.5a	6.7a	56.2a
15	15.0a	55.0a	7.5a	81.4b
30	6.3a	45.7a	5.0a	74.6ab
60	16.6a	22.4a	7.5a	63.2ab
120	9.2a	—	8.3a	57.9a

[1] Means in the same column followed by the same letter are not significantly different by Duncan's multiple range test ($P < 0.05$).

Culex salinarius showed no significant difference in percent mortality, average number of eggs/female (Table 3) and percent hatchability at dosages used in test 2. *Culex salinarius* did appear to be less susceptible than *Ae. albopictus* to the effects of ivermectin. These results correspond to those in previous studies by Tesh and Guzman (1990) that showed lower susceptibility of *Cx. quinquefasciatus* to ivermectin when compared to *Aedes* species.

Aedes albopictus also exhibited no difference in percent mortality in test 2 (Table 3). Difference in average number of eggs laid was not dosage dependent. Percent hatchability was significantly different with reduced hatchability occurring in the treatments (31–37%) as compared to the untreated eggs (57%). The analysis of ivermectin concentration in test 2 showed no ivermectin in blood prior to treatment, but analysis was not obtained for blood 4 h posttreatment due to diagnostic laboratory error.

The effects of ivermectin on mosquitoes feeding on treated dogs most likely will be minimal for most mosquito species because ivermectin has a short biological half-life (1.6 days) and because the prescribed dosage for heartworm prophylactic is approximately 6 μg/kg. *Anopheles quadrimaculatus* was the species most affected by ivermectin, and *Ae. albopictus* was somewhat less. Ivermectin use in other animals in which larger dosages are given (i.e., bovine—200 μg/kg) and the half-life is greater (i.e., bovine—2.8 days), may have greater effects on local mosquito populations.

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