# LETHAL EFFECTS OF IVERMECTIN ON *ANOPHELES QUADRIMACULATUS*

J. W. JONES, M. V. MEISCH, C. L. MEEK[1] AND W. S. BIVIN[2]

*Department of Entomology, University of Arkansas, Fayetteville, AR 72701*

ABSTRACT. Female *Anopheles quadrimaculatus* adults were blood fed on 15 mixed breed dogs 4 h after the dogs were given oral dosages of ivermectin. Dogs were divided into 5 treatment groups of 3 dogs each, at 10, 500, 1,000, 2,500 μg/kg, and untreated. Additionally, *An. quadrimaculatus* were fed on lambskin-membranes containing blood drawn from one dog in each treatment group. Mosquitoes were allowed to feed on the dogs or the lambskin-membranes and were observed for death at 24 and 48 h post-feeding. Greater than 90% mortality was recorded in all ivermectin treatment groups except at the 24 h post-feeding period with the 10μg/kg dog dose blood fed through the lambskin-membrane (65.4% mortality). The highest 2 dosages produced 100% mosquito mortality at 48 h post-feeding from either a dog or the *in vitro* system using a lambskin-membrane.

## INTRODUCTION

Avermectins have a wide range of activity against a large number of helminths and arthropods. Many publications describe the effect of avermectins on helminths and arthropods parasitic on mammals, but few studies report the effect of these compounds on mosquitoes.

Pampiglione et al. (1985) reported 100% mortality in *Anopheles stephensi* Liston 36 h after feeding on mice given 2.8 mg/kg of ivermectin, a semisynthetic avermectin. When *Aedes aegypti* (Linn.) and *Culex quinquefasciatus* Say were fed on mice given 28 mg/kg of ivermectin they exhibited about 50% mortality within 72 h of feeding. The $LD_{50}$ for *Cx. quinquefasciatus* fed on ivermectin-treated mice was 82 mg/kg. Focks et al. (1991) examined the effects of ivermectin on the reproductive rate of *Ae. aegypti.* After *Ae. aegypti* females were fed on rabbits injected with ivermectin at 10 mg/kg, the mosquitoes exhibited reduced survival and egg production when compared with females fed on control rabbits. 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 (Tesh and Guzman 1990). They reported that the $LD_{50}$s for *Ae. aegypti, Ae. albopictus* and *Cx. quinquefasciatus* fed ivermectin in human blood were 126, 208 and 698 ng/ml, respectively.

We evaluated the effects of canine blood containing selected dosages of ivermectin on freshly fed *Anopheles quadrimaculatus* Say females. Adult survival was the primary factor considered.

## MATERIALS AND METHODS

Adults of *An. quadrimaculatus* were obtained from resting stations on the University of Arkansas Rice Research and Extension Center approximately 13 km east of Stuttgart, AR (Arkansas County). Female mosquitoes were manually separated from males by chilling collection tubes in a freezer for 40 sec and then placing them on a chill table. Aliquots of 40 females were transferred by mouth aspirator to each 236-ml cardboard container (Fonda Group Inc., Union, NJ).[3] Each container was subsequently covered with nylon mesh screening. Seventy containers of mosquitoes were placed in an insulated chest for ground transportation to Baton Rouge, LA. Moistened paper toweling was added to the chest to sustain a high humidity level for mosquito survival during transportation.

Fifteen mixed breed dogs, housed at the Louisiana State University School of Veterinary Medicine, were divided into 5 groups of 3 dogs with one group acting as a control (untreated). Dogs were approximately 1 year old and weighed 19.0 ± 3.6 kg. Ivermectin[4] was diluted with Formulation B[5] and was administered orally with a 3 cc syringe at 10 μg/kg (= the prophylactic dog heartworm dosage), 500 μg/kg, 1,000 μg/kg and 2,500 μg/kg. The control dogs were given 3 cc of Formulation B. Four hours after administering ivermectin, the dogs were immobilized using 1 ml pentobarbital/2.25 kg. Blood samples were drawn from each dog, centrifuged and the su-

[1] Department of Entomology, Louisiana Agricultural Experiment Station, Louisiana State Agricultural Center, Baton Rouge, LA 70803.

[2] Division of Laboratory Animal Medicine, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA 70803.

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

[4] Ivermectin and Formulation B were supplied by Merck, Sharp and Dohme Research Laboratories, Rahway NJ.

[5] Formulation B is a solvent consisting of 60% propylene glycol and 40% glycerol formal.

pernatant placed in 3 cc EDTA tubes. Four containers, each holding 40 mosquitoes, were taped to each dog with the nylon screening of the container in contact with 4 previously shorn areas along one side of each dog (front shoulder, rib cage, hip and inside the hind leg). Mosquitoes were allowed to feed through the nylon screening for 30 min. Previous trials had indicated that 30 min would be sufficient for the mosquitoes to feed to repletion. After this period, the containers were removed, and the mosquitoes, while still in the original containers, were provided cotton balls soaked in 10% sugar water as a carbohydrate source. Blood samples were taken again and handled as before. Blood samples were packed in ice and shipped to Merck Laboratories for ivermectin concentration analysis. Mosquitoes were transported to the University of Arkansas Medical-Veterinary Entomology Laboratory in Fayetteville, AR to continue the study. Mortality readings were taken 24 and 48 h post-feeding. Data were subjected to analysis of variance and means were separated by Duncan's new multiple-range test (Dowdy and Wearden 1991).

A 50-ml aliquot of blood was taken from one dog in each treatment and control group, packed in ice and transported to Fayetteville to feed on *An. quadrimaculatus* via a natural lambskin-membrane, i.e., condom. Membrane feeding was initiated to ensure that substances on the hair or skin of the dog had not influenced the outcome of the test. The mosquitoes were collected at the same location near Stuttgart, transported to Fayetteville, and handled in the laboratory as previously described for those mosquitoes used in the dog-host test. The procedure was begun 72 h after blood was drawn from the dogs. Condoms containing blood were heated to 38°C in a water bath and then placed on the top of the cardboard containers for mosquitoes to feed. Four containers, each containing 40 mosquitoes, were used for each treatment. Mosquitoes fed on the condoms through the nylon screen as discussed previously. Mortality was determined at 24 and 48 h, and data were analyzed as previously described.

Table 1. Percent mortality of *Anopheles quadrimaculatus* fed on blood containing ivermectin[1,2]

| Dosage rates (μg/kg) | Posttreatment (hours) | | | |
|---|---|---|---|---|
| | Dogs | | Lambskin-membranes | |
| | 24 h | 48 h | 24 h | 48 h |
| 10 | 90.5Aa | 98.6Aa | 65.4Bb | 90.8Aa |
| 500 | 93.2Aa | 99.5Aa | 92.6Aa | 97.3Aa |
| 1,000 | 94.4Aa | 100.0Aa | 99.2Aa | 100.0Aa |
| 2,500 | 96.9Aa | 100.0Aa | 100.0Aa | 100.0Aa |
| Control | 1.0Bb | 4.3Ab | 1.6Bc | 3.4Ab |

[1] Means in the same row followed by the same upper case letter are not significantly different ($P \geq 0.05$ level) by DMRT.

[2] Means in the same column followed by the same lower case letter are not significantly different ($P \geq 0.05$ level) by DMRT.

Table 2. Concentrations of ivermectin in blood sampled prior to and subsequent to *Anopheles quadrimaculatus* feeding[1]

| Animal no. | Dosage rate (μg/kg) | Concentration of ivermectin (ng/ml)[2] time post-treatment | |
|---|---|---|---|
| | | 4 h | 4.5 h |
| 1 | 10 | 39 | 37 |
| 2 | 10 | 27 | 26 |
| 3 | 10 | 45 | 37 |
| Mean | | 37 | 33 |
| 4 | 500 | 191 | 157 |
| 5 | 500 | 161 | 146 |
| 6 | 500 | 205 | 175 |
| Mean | | 186 | 159 |
| 7 | 1,000 | 394 | 363 |
| 8 | 1,000 | 369 | 305 |
| 9 | 1,000 | 96 | 180 |
| Mean | | 286 | 282 |
| 10 | 2,500 | 629 | 554 |
| 11 | 2,500 | 601 | 494 |
| 12 | 2,500 | 134 | 101 |
| Mean | | 456 | 383 |
| 13 | Control | 0 | 0 |
| 14 | Control | 0 | 0 |
| 15 | Control | 0 | 0 |

[1] Initial blood samples were taken 4 h after dogs were treated.

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

## RESULTS AND DISCUSSION

Mortality rates exceeded 90% for mosquitoes fed on dogs at all rates of ivermectin at 24 h post-feeding (Table 1). Mortality in mosquitoes fed on the lambskin-membranes also exceeded 90% at all rates at 24 h post-feeding, except the 10 μg/kg treatment (65.4%. However at 48 h post-feeding, mortality at that particular treatment level increased to 90.8% and was not significantly different ($P \geq 0.05$) from the other 3 dosages. The mortality recorded at all dosages exceeded 90% in both dog-fed and the lambskin-membrane-fed groups after 48 h post-feeding. The 2 highest dosage rates resulted in 100% mortality; whereas, the control mortality never exceeded 4.3%.

We prepared individual oviposition cups for mosquitoes to measure fecundity as well as mortality but the high rate of mortality precluded any measurement of fecundity. Results from this study demonstrated much higher ivermectin activity against a mosquito species than previously reported.

The blood ivermectin concentrations recorded in the 10 μg/kg group (Table 2) are much higher than Tesh and Guzman (1990) recorded as causing egg infertility in culicine mosquitoes. The blood ivermectin concentrations at all other dosages exceeded the $LD_{50}$ recorded by Tesh and Guzman (1990) for *Ae. aegypti*. All concentrations produced very high mortality in *An. quadrimaculatus* within 48 hours.

Sigel and Baur (1987) reported that the stimulatory effects of avermectin on chick neuronal GABA receptors were additive to the ones by the barbiturate pentobarbital, if both agents were added at low concentrations. Each of these agents inhibited the stimulatory effects of the other at higher concentrations. Clearly pentobarbital had no singular effect as demonstrated by controls.

Numerous questions exist regarding the effect of ivermectin on local mosquito populations. Boluses for cattle can extend the time of ivermectin availability in the blood; however, ivermectin is normally administered only monthly in canines and has a short biological half-life (1.6 days). Nevertheless, the use of ivermectin as an anthelminthic has increased dramatically in canines and it is likely that ivermectin is often imbibed during blood meal acquisitions by female mosquitoes. Its effect on mosquito fecundity as well as survival is an area requiring further evaluation.

## ACKNOWLEDGMENTS

We are grateful to Richard Endris of Merck, Inc. for his assistance in the project and to David Fink, Pierre DeMontigny and Jessica Cha of Merck Laboratories for conducting the blood sample analysis.

## REFERENCES CITED

Dowdy, S. and S. Wearden. 1991. Statistics for research, 2nd ed. John Wiley & Sons, New York.

Focks, D. A., R. E. McLaughlin and S. B. Linda. 1991. Effects of ivermectin (MK-933) on the reproductive rate of *Aedes aegypti* (Diptera: Culicidae). J. Med. Entomol. 28:501–505.

Pampiglione S., G. Majori, G. Petrangeli and R. Romi. 1985. Avermectins, MK-933 and MK-936, for mosquito control. Trans. R. Soc. Trop. Med. Hyg. 79:797–799.

Sigel, E. and R. Baur. 1987. Effect of Avermectin B1a on chick neuronal gamma-aminobutyrate receptor channels expressed in *Xenopus* oocytes. Mol. Pharmacol. 32:749–752.

Tesh, R. B. and H. Guzman. 1990. Mortality and infertility in adult mosquitoes after the ingestion of blood containing ivermectin. Am. J. Trop. Med. Hyg. 43:229–233.