BioAssay

CHEMICAL CONTROL

Susceptibility of *Aedes aegypti* (L.) (Diptera: Culicidae) Immature Forms to Ivermectin

Cassiano S. Rosa[1], Daniel S. Albeny[2], Livia M. S. Ataíde[3], Marco A. P. Horta[4] & Evaldo F. Vilela[5]

[1]*Entomology Graduate Program Dept. Animal Biology-UFV, 36570-000 Viçosa MG Brazil - cassianosr@yahoo.com.br*
[2]*Entomology Graduate Program Dept. Animal Biology-UFV, 36570-000 Viçosa MG Brazil - daniel@insecta.ufv.br*
[3]*Entomology Graduate Program Dept. Animal Biology-UFV, 36570-000 Viçosa MG Brazil - livia@insecta.ufv.br*
[4]*Instituto Superior de Ciências da Saúde. Av. Barão Homem de Melo, 4324. Belo Horizonte, MG. 30450-250. entomologica@gmail.com*
[5]*Dept. Animal Biology-UFV, 36570-000 Viçosa MG Brazil - evilela@ufv.br*

BioAssay: 6:6 (2011)

Suscetibilidade de formas imaturas de *Aedes aegypti* (L.) (Diptera: Culicidae) à ivermectina

RESUMO - O combate ao mosquito transmissor do dengue, *Aedes aegypti* (L.), tem sido um dos principais meios de se evitar epidemias dessa doença e para alcançar esse objetivo, o uso de larvicidas tem aumentado consideravelmente. Atualmente o larvicida mais utilizado pelos programas de saúde governamentais no Brasil é o temephos, entretanto, já existem registros de populações de *A. aegypti* resistentes a esse fármaco, destacando-se a necessidade de se pesquisar novos inseticidas. A ivermectina é uma droga antiparasítica muito utilizada na pecuária no combate a nematóides e artrópodes por apresentar baixa toxicidade a vertebrados, quando aplicada em pequenas doses. Considerando sua eficiência no controle de alguns artrópodes, o objetivo desse trabalho foi testar o efeito deste fármaco em larvas de 3º e 4º ínstares de *A. aegypti*. As larvas foram expostas à ivermectina durante 5, 15, 30, 60 e 1440 minutos, nas concentrações de 1, 5 e 10 ppm de solução de ivermectina. Foi feita observação 24 horas após o contato do inseto com a droga e observada a taxa de mortalidade. Observou-se uma correlação positiva entre a concentração de ivermectina e a taxa mortalidade das larvas, independente do tempo em que estas ficaram em contato com a droga. Através da análise dos resultados obtidos, pode-se concluir que o aumento da dose de ivermectina aumenta a mortalidade das larvas de *A. aegypti*, demonstrando assim, a eficácia do fármaco utilizado.

Palavras-chaves: *Aedes aegypti*, Dengue, ivermectina

ABSTRACT - Several mosquito populations have already exhibited resistance to various insecticides, a situation that imposes many problems in vector control programs in many countries. Several studies have recently reported a decrease in the persistence of temephos, the main larvicide used to control *Aedes aegypti* in Brazil, suggesting that mosquito populations have acquired resistance to this organophosphate in several states. Dengue is one of the most important arthropod-borne diseases in the world and in Brazil thousands of dengue cases are notified annualy. In order to verify the effects of ivermectin, a semi-synthetic drug widely used for treatment of livestock parisitic diseases, on *Aedes aegypti* larvae of 3rd and 4th instar, the insects were submitted to concentrations of 1, 5 and 10 ppm of ivermectin solution during 5, 15, 10, 60 and 1440 minutes. After 24 hours of observations, mortality rates were scored. Loss of mobility, progressive paralysis and high mortality of larvae were recorded. Observations suggest that few minutes of contact with the insect is sufficient to cause intoxication. Increases in ivermectin concentration cause a progressive mortality in *A. aegypti* larvae. The values presented in the present study indicate that chemical treatment using ivermectin may be an alternative to the use of ivermectin in dengue control programs.

Key-words: *Aedes aegypti*, Dengue, ivermectin

Dengue is the most important arthropod-borne viral disease of public health significance. Today, the geographic distribution includes more than 100 countries worldwide. The World Health Organization (WHO 1981) estimates that more than 2.5 billion people are at risk of dengue infection. Half the world's population lives in countries endemic for dengue, underscoring the urgency to find solutions for dengue control (Guha-Sapir & Schimmer 2005). Since 1986 when dengue fever was introduced to Brazil, the disease has been detected every year (Ministério da Saúde 2007). During the 90's thousands of cases of dengue were recorded per year. The last epidemic peak occurred in 2002 when a new virus DEN-3 was found and 794,219 cases were notified mainly in the state of Rio de Janeiro. In 2006, 345,922 cases were notified mainly in southeastern and northeaster regions with 76 death cases. In Brazil, most of the notifications for dengue fever occur between January and March, a period of high rainfall and high temperatures which provides suitable conditions for the development of mosquito *A. aegypti* (Ministério da Saúde 2007).

Since the initial producing of DDT at the beginning of the 40's a great number of synthetic pesticides have been used for control of insect vectors of diseases. Development associated with the environmental concern is one of the great challenges for the new century. Since the last decades, efforts have been directed to production of insecticides with low toxicity for vertebrate species, low capacity of accumulation in the environment and high specificity for enemy species. Whereas the raising of resistance in several target species have functioned as pressure in developing new effective drugs. Several insecticides have been tested to control mosquito populations in order to reduce the transmission of many diseases by these insects. Organophosphates, carbamates, pyrethroides and DDT are still frequently used in Brazil for control of insects.

Mosquitoes *A. aegypti* have exhibited resistance to various insecticides, a situation that imposes many problems in vector control programs in many countries (Lima *et al.* 2003). In Brazil, public health programs have used mostly organophosphates in the control of *A. aegypti*, however, this procedure has not prevented the appearance of several dengue epidemics (Pontes & Ruffino-Netto 1994). Also, several studies have recently reported a decrease in the persistence of temephos, the main larvicide used to control *A. aegypti* in Brazil, suggesting that mosquito populations have developed resistance to this organophosphate in several states (Carvalho *et al.* 2004)

Ivermectin is a group of macrocyclical lactones, derived from 22,23-dihydro avermectin B1, with low water solubility that is produced by *Streptomyces avermitilis*, being largely used as anti-parasitic in the domestic animals (Benz *et al.* 1989). Previous studies have shown that the avermectins are lethal for immature and adult stages of some insect orders and highly efficient for the control of larvae of *Aedes*, *Anopheles* and *Culex* (Freitas *et al.* 1996, Alves *et al.* 2004) when exposed to low concentrations of ivermectin. It is not necessary its ingestion, because the contact with the same is effective in the majority of the cases (Strong 1993). The aim of this study is to assess the effects of different concentrations of ivermectin on larval instars of *A. aegypti*.

Material and Methods

The study was conducted in the city of Coronel Fabriciano, Minas Gerais state, Brazil, in June 2004. The municipality is extended in an area of 221 km^2 with 103,724 inhabitants, and has a historic in dengue notifications, with thousands of people affected by the illness in the last years. The warm temperature, high rainfall and the geographic position of the city has created an ideal location for breeding sites of *A. aegypti* populations assuring their survival for all the year.

Samples of *A. aegypti* were collected by using ovitraps prepared with black plastic jars filled with hay infusion. The number of ovitraps installed followed a norm established by FUNASA (1999) and was based on the number of buildings in each municipality (an indirect measure of the population density): 60,000 buildings, 100 ovitraps; 60,000-120,000 buildings, 150 ovitraps; 120,000-500,000 buildings, 200 ovitraps; 500,000 buildings, 300 ovitraps. Field collection of eggs was done from January to March 2004. After five days exposed, the ovitraps were collected and taken to the laboratory where the presence of eggs in each ovitrap was then scored.

Positive ovitraps were immersed in dechlorinated water to induce larval hatching. After 24 hours, larvae were transferred to rectangular basins containing two liters of dechlorinated water. Dog food was supplied daily to feed the larvae. Pupae and emerged adults were transferred to plastic jars. Mosquito identification followed the literature of Consoli & Oliveira (1994). Only *A. aegypti* mosquitoes were kept for bioassays. These were transferred to square plastic cages (60 cm per side) and fed on 10% solution of honey with distilled water and later fed on anesthetized guinea pigs three days after emergence of the adults. Three days after the blood meal, eggs were collected for three days in small plastic cups containing wet filter paper. Paper strips containing the eggs were then allowed to dry in an insectary and served as the source of F1 mosquitoes for the bioassays.

Ivermectin resistance bioassays were performed with F1 larvae, according to the WHO recommended procedure and parameters (WHO 1981). Groups of 3 and 4 instar of *A. aegypti* larvae were placed to 50 mL plastic recipients containing a solution of Ivermectin 1% [(22,23-dihidroavermectin B1 (Ivomec®)] in different concentrations of 1, 5 and 10 ppm during different times of exposition (5, 15, 30, 60 and 1440 minutes). Control groups were placed in recipients containing just dechlorinated water. One hundred larvae were used for each tested concentration of ivermectin, and 20 larvae for each time of exposition and the control test. The bioassays were repeated two times for each group.

After the exposure time, larvae were washed and transferred to other plastic recipients containing dechlorinated water. No food was offered to larvae during the experiment time. The experiments were conducted by adapting WHO methodology used to test insecticides in mosquito larvae (WHO 1981). *A. aegypti* larvae were examined in a stereomicroscope 24 hours after starting the experiment.

Mortality was scored 24 hours after the beginning of the test. In all cases, the resistance/susceptible status of mosquito populations was evaluated according to WHO criteria (O. M. S. 1976). Mortality greater than 98% indicates susceptibility,

mortality less than 80% defines resistance, and mortality between 80% and 98% is suggestive of an incipient altered susceptibility, indicating the need for surveillance of the corresponding population. All the analyses were carried out using generalized linear modelling and Poisson errors with log link, and were performed under "R" software (R Development Core Team 2006) followed by residual analyses to verify error distribution and the suitability of the models employed, including checks for over-dispersion.

Results

The observations suggest a potent effect of ivermectin on *A. aegypti* larvae even when these insects have a low time of exposition to drug. The time by which the larvae remained in contact with the drug showed no significance when compared to mortality rates. The analyses have shown that the time of exposition to ivermectin does not affect the larvae mortality ($F = 29.25$; d.f. = 1, 43; $P < 0.0001$) (Table 1). This fact emphasizes the powerful effect of ivermectin and suggests that low times of exposition are able to cause reasonable results. Observations show that the concentration of ivermectin positively affects the mortality of *A. aegypti* larvae ($F = 29.25$; d.f. = 1, 43; $P < 0.0001$) (Figure 1). Concentrations of drug above 5 ppm increase the chance of mortality mainly for larvae exposed to 30 minutes in contact with ivermectin. When larvae are submitted to 1 ppm concentrations, mortality rates average around 50%. When exposed to 10 ppm concentrations, observed mortality rates are higher than 75% for all times of contact with the drug.

Table 1. Number of dead larvae of *Aedes aegypti* when submitted to different concentrations of ivermectin solution (1, 5 and 10 ppm).

	Time of exposure (min)					Concentration rate (ppm)
	1140	60	30	15	5	
N. of dead larvae	19	20	15	14	16	10
	16	15	17	17	17	10
	13	18	15	18	17	10
	15	8	11	9	5	5
	18	19	17	15	13	5
	10	16	17	14	14	5
	13	5	4	4	8	1
	15	15	13	11	10	1
	4	13	9	7	8	1

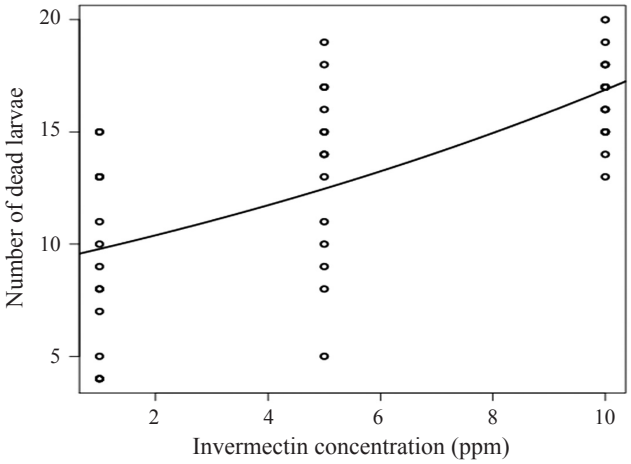

Figure 1. Mortality of *Aedes aegypti* larvae when submitted to 1 ppm, 5 ppm e 10 ppm of ivermectin ($F = 29.25$; df = 1, 43; $p < 0.0001$).

Discussion

For all concentrations tested during the experiment, the *A. aegypti* larvae affected by ivermectin display ataxia and progressive paralysis. Ivermectin also caused paralysis in *Culex quinquefasciatus* larvae, as observed by Alves *et al.* (2004). The high mortality rate found for larvae submitted to ivermectin was in agreement with results found for *Cx. quinquefasciatus* larvae (Alves *et al.* 2004), for *Chrysomya bezziana* and *Calliphora vomitoria* (Strong 1993) who found inhibition of pupation and adult development in exposed to sub-lethal doses of ivermectin. Previous results have proposed that the times of generation for mosquitoes exposed to ivermectin have shortened about 1 day its development from egg to adult, suggesting that the ivermectin exposure of parental generation may cause a rapid development to avoid the environment stress (Vianna *et al.* 1996).

Ivermectin can bind to specific ion canals directly, following a disturbance of the electric balance (Campbell 1985). However, action of the ivermectin may be associated with neurotransmitter release in conjunction with the GABA antagonist, probably contributing for excitatory signals of poisoning (Alves *et al.* 2004). In the present study, the majority of the larvae of *A. aegypti* exposed to concentrations of 1, 5 and 10 ppm of ivermectin solution presented ataxia, followed by death, suggesting binding of this insecticide to the GABA with consequent lethal action for *A. aegypti*, which agreed with results obtained by Freitas *et al.* (1996) and Alves *et al.* (2004) for *Cx. quinquefasiatus*. Even those larvae who did not die when exposed to low concentrations of ivermectin might lately suffer the consequences of drug which would be expressed at adult body.

Avermectins in soil and aquatic environments show a rapid degradation which is accelerated by exposure to sunlight, reducing half-lives to 0.5 days or less (Halley *et al.* 1993). Although Freitas *et al*. (1996) reported that when kept in glass jar for at least one month under laboratory conditions, at room temperature in absence of direct sunlight, ivermectin solutions of 1, 5 and 10 ppm remained active

against mosquito larvae.

The values presented in the present study indicate that chemical treatment using ivermectin is viable. The results confirm the need for preventive strategies and alternative control methods that might diminish the selection of resistance after the massive use of temephos in dengue control programs. The constant use of pesticides worldwide has brought a general tolerance for these chemicals and the outcome of resistance. In Brazil several authors have reported the appearing of resistance of *A. aegypti* to temephos, the most used insecticide by health programs from municipalities (Carvalho *et al.* 2004). Ivermectin rises as an alternative for the future substitution of temephos in mosquito control programs whereby it has shown effectiveness in killing *A. aegypti* and *Cx. quinquefasciatus* larvae under laboratory conditions. Even some other drugs used by mosquito control programs as cypermethrin has already shown signs of resistance in Brazil (Luna *et al.* 2004), suggesting a need for immediate replacement of these insecticides by some other product of chemical or biological origin.

References

Alves, S.N., J.E. Serrão, G. Mocelin & A.L. Melo. 2004. Effect of ivermectin on the life cycle and larval fat body of *Culex quinquefasciatus*. Braz. Arch. Biol. Technol. 47: 433–439.

Benz, G.R. Roncalli & S. Gross. 1989. Use of ivermectina in cattle, sheep, goats, and swing. In: Ivermectin and Abamectin (ed. Campbell, W. C.), Springer-Vertag New York. pp. 215–229.

Campbell, W.C. 1985. Ivermectin: an update. Parasit. Today, 1: 10–16.

Carvalho, M.S.L., E.D. Caldas, N. Degallier, P.T.R. Vilarinhos, L.C.K. Souza, M.A.C. Yoshizawa, M.B. Knox & C. Oliveira. 2004. Susceptibilidade de larvas de *Aedes aegypti* ao inseticida temefós no Distrito Federal. Rev. Saúde Públ., 38: 623–629.

Consoli, R.A.G.B. & R.L. Oliveira. 1994. Principais mosquitos de importância sanitária no Brasil. Fiocruz.

Freitas, R.M., M.A. Faria, S.N. Alves & A.L. Melo. 1996. Effects of ivermectin on *Culex quinquefasciatus* larvae. Rev. Inst. Med. Trop. São Paulo, 38: 293–297.

FUNASA 1999. Reunião técnica para discutir status de resistência de *Aedes aegypti* e definir estratégias a serem implantadas para monitoramento da resistência no Brasil. Brasília, Brazil.

Guha-Sapir, D. & B. Schimmer. 2005. Dengue fever: new paradigms for a changing epidemiology. Emerging Themes in Epidemiology, 2: 1-10.

Halley, B., W. Vandenhuvel & P. Wislocky. 1993. Environmental effect or the usage of avermectins in livestock. Vet. Parasit., 48: 109–125.

Lima, J.B.P., M.P. Cunha, R.C. Silva-Jr, A.K.R. Galardo, S.S. Soares, I.A. Braga, R.P. Ramos & D. Valle. 2003. Resistance of *Aedes aegypti* to organophosphates in several municipalities in the state of Rio de]aneiro and Espírito Santo, Brazil. Am. J. trop. med. hyg, 68: 329–333.

Luna, J., M. Martins, A. Anjos, E. Kuwabara & M. Navorro-Silva. 2004. Susceptibility of *Aedes aegypti* to temephos and cypermethrin inseticides, Brazil. Rev. Saúde Públ. 38: 842–843.

Ministério da Saúde: SECRETARIA DE VIGILÂNCIA EM SAÚDE. 2007.Balanço Dengue Janeiro a Julho de 2007. Brasília, Brazil.

O.M.S. 1976. Resistencia de vectores y reservorios de enfermidades a los plaguicidas. Informe del Comité de Expertos de la OMS en Insectcidas 585, Organizacion Mundial de la Salud, Ginebra.

Pontes, R.J.S. & Ruffino-Netto, A. 1994. Dengue em localidade urbana da região sudeste do Brasil: aspectos epidemiológicos. Rev. Saúde Públ., 28: 218–227.

R Development Core Team. 2006. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0. http://www.R-project.org

Strong, L. 1993. Ovewrview: the impact of avermectin on pastureland ecology. Vet. Parasit., 48: 3–17.

Vianna, E.E.S., P.R.P. Costa & P.B. Ribeiro. 1996. Oviposiição e longevidade de adultos do *Culex quinquefasciatus S*ay, 1823 (Diptera: Culicidae) em condições ambientais, em Pelotas, RS. Rev. Bras. Parasitol. Vet., 5: 47–52.

WHO 1981. Instructions for determining the susceptibility or resistance of mosquito larvae to insecticides: report of the WHO Expert Committee on Resistance of Vectors and Reservoirs of Diseases to Pesticides.

Received: 22/XI/2007 Accepted: 28/VI/2009 Published: 30/VIII/2011 Available online: www.bioassay.org.br/ojs/index.php/bioassay/article/view/102