# EFFICACY OF A SUSTAINED-RELEASE METHOPRENE FORMULATION ON POTENTIAL VECTORS OF RIFT VALLEY FEVER VIRUS IN FIELD STUDIES IN KENYA

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Many *Aedes* spp. and *Culex* spp. mosquitoes with larval habitats in ground pools have been incriminated as potential enzootic or epizootic vectors of Rift Valley fever (RVF) virus in Africa (Meegan and Bailey 1988). Linthicum et al. (1984) reported that 6 of the 8 species of mosquitoes collected in one flooded grassland depression, known as a dambo, in a RVF epizootic area in Kenya were associated with RVF virus ecology. After the inundation of dambos, drought-resistant *Ae.* spp. eggs hatch and their immatures are the first mosquitoes collected in these habitats. Subsequently, multiple generations of *Cx.* spp. invade the flooded dambos and their adults may continue to emerge for as long as the habitat remains flooded.

Specific control measures employed against immature ground pool mosquitoes in RVF enzootic/epizootic areas could serve as an important and effective source-reduction management strategy for limiting RVF virus introduction into susceptible domestic animals and possibly curtailing RVF outbreaks. Some insect growth regulators (IGRs), have been shown to be highly selective in controlling mosquitoes (Miura and Takahashi 1973, 1975) and are relatively long-lasting and effective at low application rates. In this study, a sustained release-formulation of methoprene in Altosid® pellets was used to evaluate its effect on ground pool mosquitoes found in a dambo system in a RVF virus enzootic/epizootic area in Kenya.

The dambo system studied in this field trial was located approximately 8 km SSE of Ruiru, Thika District, Central Province, Kenya (1°13′S;36°58′E) at an elevation of approximately 1500 m. The dambo was located in an area of scattered *Acacia* spp. trees and bushes on grasslands along the Kiu River. It exhibited the typical zonal differentiation described by Mackel (1974) and Linthicum et al. (1983) with predominantly sedge *(Cyperus immensus* C.B. Clarke) in the central area and the grass *Digitaria abyssinica* (A. Richard) Stapf in the remainder of the site.

An area of approximately 5000 m$^2$ was artificially flooded by pumping water from the Kiu river using 2 gasoline-operated pumps. The flooded region was maintained at a constant level for 30 days. Water depth varied from 0.2 to 0.4 m. Immediately after flooding, 2 separate treatment sites within the flooded dambo were formed by constructing a trenched barrier lined with polyurethane sheets to prevent water flow in or out of these sites. The portions of the dambo selected for use as treatment sites were similar in terms of plant species, species distribution and density, and topography, to the remainder of the flooded dambo, which served as the control area.

Altosid pellets were applied by hand to the first treatment site (467 m$^2$) at a rate of 5.6 kg/ha (0.22 kg Al/ha) the day after flooding. Altosid pellets were applied at the same rate to the second treatment site (100 m$^2$) 3 days after flooding.

Dip collections were made daily, starting day 1 after flooding, in both treatment sites and within the control area, by sampling with 0.47-liter dippers. Fifty samples were taken by dipping at equal intervals along each of 2 lines transecting each of the sites. All immatures were separated by stage, counted and replaced in the dambo.

To evaluate the effect of methoprene treatment on larval populations, an estimate of the daily survival rate of larvae at different periods of the study was made using the methodology described by Linthicum et al. (1985). Daily probability of larval survival within treatment and control areas for the different time periods was compared by generating 95% confidence intervals for the regression slopes and by testing slopes for parallelism by analysis of variance (ANOVA) (SAS/STAT 1988).

When pupae began to appear in the dambo, and daily thereafter, samples were collected and counted for the 2 treatment and control sites from regions other than the daily transect lines, to avoid affecting counts in these areas, and placed in either 0.3-m$^3$ or 1-m$^3$ cages submerged in up to 0.3 m of water. To monitor treatment effectiveness on pupal survival for different species, adults which emerged into the cages were collected, identified and counted daily until no

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live individuals remained. The percent mortality of pupae was determined separately for one generation of *Ae.* spp. and multiple generations of *Cx.* spp. Multiple $\chi^2$ tests of mortality within different time periods were performed (SAS/STAT 1988).

Eggs of *Ae. circumluteolus* (Theobald), *Ae. cumminsii* (Theobald), *Ae. dentatus* (Theobald), *Ae. mcintoshi* Huang, *Ae. sudanensis* (Theobald), and *Ae. unidentatus* McIntosh hatched soon after flooding in both treatment and control sites. First-stage larvae were collected between 1 and 4 days after flooding. Between 6 and 10 days after flooding *Aedes* spp. pupae were placed in emergence cages in the control (n = 2,148) and treatment ($n$ = 1,000) areas. All 6 *Aedes* spp. were collected in the emergence cages in the control area between 8 and 12 days after flooding. *Ae. mcintoshi* was the most common, representing 76.1% of the total *Aedes* spp. *Ae. circumluteolus* and *Ae. dentatus* represented 19.2% and 4.2%, respectively, of the total *Aedes* spp. First stage *Cx. antennatus* (Becker), *Cx. duttoni* Theobald, *Cx. theileri* Theobald, *Cx. tigripes* De Grandpre and De Charoy and *Cx. zombaensis* Theobald larvae were first collected in treatment and control sites starting 6 days after flooding. Between 11–26 days after flooding *Culex* spp. were placed in emergence cages in the control ($n$ = 1,267) and treatment ($n$ = 1,775) areas. Adult *Culex* species were collected in the emergence cages in the control area between 13 and 30 days after flooding. Of the *Culex* spp., *Cx. antennatus* was the most commonly collected (63.3%), followed by *Cx. zombaensis* (17.1%) and *Cx. duttoni* (16.1%). Three *Anopheles coustani* Laveran specimens were collected in the emergence cages 16 and 20 days after flooding.

Larvae in both treatment sites appeared to have lower survival rates than in the control area during the first 3 weeks after flooding. However, only survival of larvae in the area treated 1 day after flooding during the second week after flooding was significantly lower (ANOVA; $P$ = 0.04) than the control area. There was no significant difference between survival of larvae in treatment and control areas between 22 and 28 days after flooding.

The efficacy of Altosid pellets against pupae of the 6 *Aedes* spp. and 5 *Cx.* spp. is shown in Table 1. Altosid pellets totally prevented the emergence of all species of *Aedes* treated as either first (day 1 after flooding) or third (day 3 after flooding) stage larvae. When compared to the percent mortality of the *Aedes* in the control area, the effect of treatment was highly significant ($\chi^2$; df = 1; $P < 0.001$). Adult emergence of the *Culex* spp. was also prevented during the second week after flooding in both treatment sites. Residual activity, as evidenced by a high pupal mortality rate (88%), was observed during the third week after flooding in the area treated 3 days after flooding. The data were also suggestive of some limited residual effect during the fourth week after flooding, when mortality of *Culex* spp. pupae appeared to be higher than in the control area, although not significantly higher ($\chi^2$; df=1; $P$ = 0.09).

The number of immatures collected/dip and the succession of *Aedes* spp. followed by *Culex* spp. observed in this study were similar to those reported by Linthicum et al. (1984) in a naturally flooded dambo. Transovarially infected *Aedes* spp. may be responsible for introducing RVF virus into susceptible vertebrate amplifying hosts. Controlling *Aedes* spp. emergence

Table 1. Efficacy of Altosid® pellets against pupae of *Aedes* spp. and *Culex* spp.

| Species | Area | Percent mortality (number dying/total number sampled) of pupae for different weeks (days) after flooding[1] | | |
|---|---|---|---|---|
| | | Week 2 (8–14) | Week 3 (15–21) | Week 4 (22–28) |
| *Aedes* spp. | Control | 32 (687–2148)a | — | — |
| | Treated 1 day after flooding | 100 (800–800)b | — | — |
| | Treated 3 days after flooding | 100 (200/200)b | | |
| *Culex* spp. | Control | 45 (459–1025)c | 46 (75–162)a | 45 (45/100)a |
| | Treated 1 day after flooding | 100 (252/252)b | 41 (41/100)a | 47 (47/100)a |
| | Treated 3 days after flooding | 100 (1064–1064)b | 88 (140/159)b | 57 (57/100)a[2] |

[1] Percents within a column followed by different letters are significantly different ($P < 0.001$); multiple comparison $\chi^2$ test.

[2] When compared to check area $P = 0.09$; $\chi^2$ test.

from flooded dambos might prevent the initiation of an epizootic even if secondary *Culex* vectors were available to transmit the virus. A single treatment of Altosid pellets effectively blocked both *Aedes* and *Culex* adult emergence for at least 2 weeks after flooding in this study. If all flooded-dambo, immature mosquito habitats could be given one Altosid treatment, RVF transmission might be interrupted even if subsequent generations of *Culex* spp. emerged. However, if some dambo habitats were not treated, or if a fluctuating water level resulted in a new emergence of *Aedes* spp., or if RVF virus were to be introduced from adjacent areas, multiple IGR treatments would be necessary as long as vector habitats remained flooded.

The extended residual activity of Altosid pellets we observed after delaying treatment until *Aedes* larvae were in the third stage suggests that the intervals for multiple treatments could be longer than every 2 weeks. Further studies should be conducted to evaluate conditions required for control over extended periods of flooding, to include evaluation of both lower and higher doses of methoprene.

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