EGG HATCHING OF *AEDES* MOSQUITOES DURING SUCCESSIVE FLOODINGS IN A RIFT VALLEY FEVER ENDEMIC AREA IN KENYA

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ABSTRACT. Floodwater *Aedes* breeding habitats in central Kenya were sequentially flooded to determine the numbers of mosquito eggs hatching during each flooding. Approximately 90% of the larvae sampled during 4 floodings emerged during the initial flooding. The number of *Aedes* eggs hatching during the second flooding was lowest of all 4 floodings, and no significant differences in the amount of egg hatching during floodings 3 and 4 were seen. Unhatched *Aedes* eggs were present in soil samples collected after the final flooding. The possible implications of these findings with regard to Rift Valley fever virus control are discussed.

Rift Valley fever (RVF) virus causes disease in domestic animals and humans. During interepizootic periods, the virus is thought to survive in Kenya in transovarially infected *Aedes mcintoshi* Huang eggs (Linthicum et al. 1985). Natural RVF viral infections have been found in the adults of many other mosquito species; the epidemiology of RVF has been recently reviewed (Meegan and Bailey 1988).

Floodwater *Aedes* spp. eggs enter a state of diapause that is broken when the embryonated eggs are submerged in water of reduced oxygen content (Gjullin et al. 1941); however, not all eggs hatch uniformly in response to submersion in deoxygenated water, and some require repeated exposures before hatching. Most *Aedes vittatus* (Bigot) eggs from mud samples collected in Nigeria hatched during the second and third flooding, and none hatched after a sixth flooding (Service 1970). More than 50% of *Aedes detritus* (Haliday) eggs hatched during floodings 4–6 when mud samples containing these eggs were soaked 13 times (Service 1968). Cooney et al. (1981) obtained 98–99% hatching of *Aedes vexans* eggs in laboratory tests in which known numbers of eggs were placed in beakers, covered with various depths of soil and flooded to stimulate hatching; only the initial flooding was performed. Tree hole debris collected by Buxton and Breland (1952) was flooded 13 times, and from this, *Aedes triseriatus* (Say) was collected 12 times, and *Aedes zoosophus* Dyar and Knab was recovered 5 times.

Mosquito control efforts directed against immature stages of floodwater *Aedes* spp. in Kenya may provide an effective vector-reduction strategy for limiting RVF virus introduction into susceptible animals, thus curtailing RVF outbreaks. The potential for use of a sustained-release methoprene formulation to interrupt both the enzootic and epizootic cycles of this disease was demonstrated (Linthicum et al. 1989). However, specific information regarding the frequency and occurrence of expected egg hatch of floodwater *Aedes* spp. during the initial and subsequent flooding in these areas is unknown. The main breeding habitats for floodwater *Aedes* spp. associated with RVF virus are "dambos" or shallow, streamless, grassland depressions associated with river drainage systems subject to seasonal flooding (Mackel 1974). To implement vector-control methods effectively against immature mosquito stages, the number of expected egg hatching during successive floodings of dambos must be known. The objective of this study was to determine the number of *Aedes* spp. eggs hatching in floodwater *Aedes* spp. after floodings of dambos in a RVF endemic zone.

The study was conducted during the dry season from January to March 1989 at a dambo system located on the west bank of the Kiu River approximately 8 km SSE of Ruiru, Thika District, Central Province, Kenya (1° 13′S; 36° 58′E, altitude 1,500 m). During 3 days of the study period, there was an accumulation of 109 mm of rainfall and no natural flooding of the dambo occurred. Two distinct areas, 180 m^2 each, within the dambo system were formed by constructing a trenched barrier lined with polyurethane sheets to prevent water flow in or out of these areas (Fig. 1). The 2 areas, separated by ca. 25 m, were similar in terms of plant species, primarily the sedge *Cyperus immensus* (C. B. Clarke), and the grass *Digitaria abyssinica* (A. Richard) Stapf. The sites were flooded with river water and maintained at a constant level of approximately 0.3 m for 5 days. Dip collections (0.47-liter dippers) were made daily for 4 days, starting day 1 after flooding. Fifty samples were

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taken by dipping at equal intervals along 2 transects in each of the sites. Immatures collected were separated by stage, counted and replaced in the dambo. At the end of the fifth day of flooding, the sites were drained of water by pumping from the sites back into the river, thus preventing adult emergence and precluding replenishment of *Aedes* eggs in the study sites. Sites were allowed to dry for 2–3 wk between each of 4 floodings, and no other *Aedes* habitats in the region were flooded during this study period to minimize extraneous *Aedes* oviposition in the study area. Light-trap collections were made nightly during each flooding period by placing solid-state, army miniature light traps (John W. Hock Co., Gainsville, FL) near the boundary of the flooded sites. Before each flooding and after the final flooding, a variable number of soil samples were taken from each site; mosquito eggs were extracted by the method of Horsfall (1956).

Egg hatching occurred in significantly (ANOVA, Tukey's Studentized range test; $P < 0.05$) larger numbers during the initial flooding compared with later floodings in each site (Table 1). Approximately 90% of the total larvae collected hatched in both sites were recovered during the first flooding. The number of *Aedes* eggs hatching during the second inundation was lowest of all 4 floodings in site 1. No significant differences in the number of larvae were seen in floodings 3 and 4.

Significantly ($P < 0.05$) more *Aedes* spp. eggs were recovered from site 1 before the first flooding as compared with all later floodings (Table 2). Although site 2 egg recovery data showed a trend towards reduced numbers of eggs after floodings 2, 3 and 4, no significant differences were seen. The larval count and egg-collection data demonstrate that egg hatching during the initial flooding of *Aedes* spp. eggs in this dambo habitat was large in comparison to successive floodings. Similarity in data collected during floodings 2–4 indicate that a small percentage of the initial number of eggs hatched in response to each reflooding. Viable eggs remained in the soil after 4 floodings. This is based on experiments in the laboratory. Light-trap collections made during this study yielded no *Aedes* specimens, so it is unlikely that new eggs were oviposited in the study areas between floodings. Quantitative rearings of immatures from study sites were not attempted to avoid possible misinterpretations that could arise from differential survival rates among species during artificial rearing manipulations. Previous studies in this area documented the presence of 7 species of floodwater aedine mosquitoes: *Aedes mcintoshi* Huang, *Ae. dentatus* (Theobald), *Ae. circumluteolus* (Theobald), *Ae. cumminsii* (Theobald),

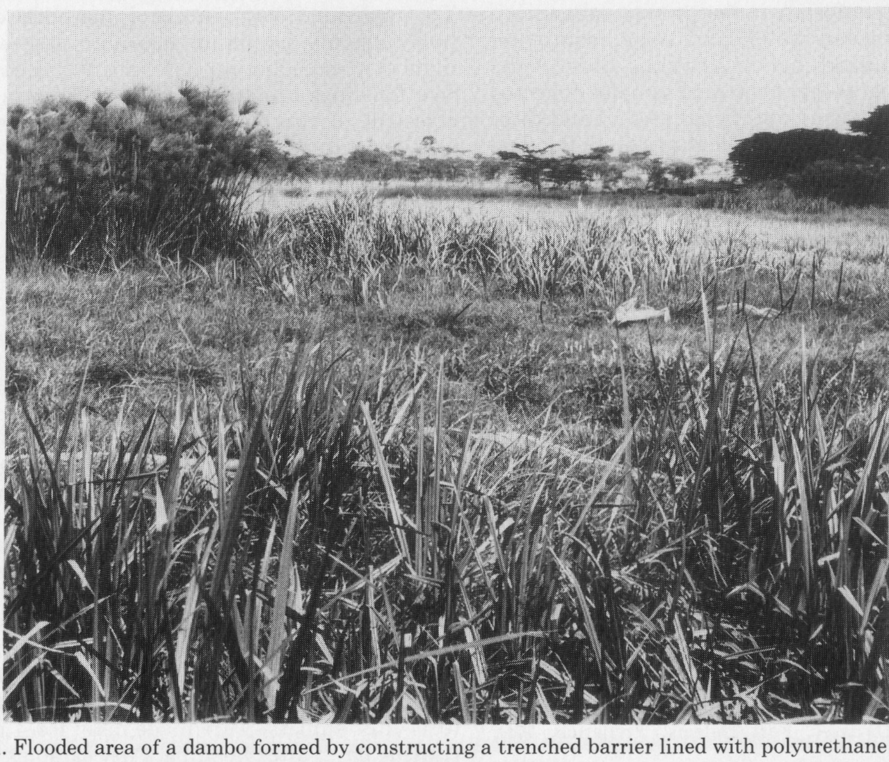

Fig. 1. Flooded area of a dambo formed by constructing a trenched barrier lined with polyurethane sheets.

Table 1. Mean daily number of *Aedes* larvae collected per dip in each site during 4 sequential floodings.

	Site 1		Site 2	
Flooding	Mean ±SE no. larvae[1]	% of total[2]	Mean ±SE no. larvae[1]	% of total[2]
1	230 ± 21	92a	150 ± 16	89a
2	5 ± 1	2b	8 ± 2	5b
3	8 ± 1	3c	6 ± 1	4b
4	8 ± 1	3c	4 ± 1	2b

[1] Number of dips = 100.
[2] Percents within a column followed by different letters are significantly different based on Tukey's Studentized range test ($P < 0.05$).

Table 2. Number of *Aedes* spp. eggs recovered from soil samples collected in study areas sequentially flooded.

	Site 1		Site 2	
Flooding	Mean ±SD no. eggs[1]	% of total[2]	Mean ±SD no. eggs[1]	% of total[2]
Prior to 1	555 ± 383	86a	104 ± 84	61a
Prior to 2	17 ± 11	3b	10 ± 9	6b
Prior to 3	27 ± 27	4b,c	41 ± 24	25b
Prior to 4	35 ± 39	5b	12 ± 17	7b
After 4	13 ± 24	2c	2 ± 4	1c

[1] Number of samples were 12 prior to the first and fourth flooding, 6 prior to second and third flooding and 50 after the fourth flooding.
[2] Percents within a column followed by different letters are significantly different ($P < 0.05$) based on a rank-sum test.

Ae. unidentatus Mcintosh, *Ae. quasiunivittatus* (Theobald) and *Ae. sudanensis* (Theobald). The most common species in this area are *Ae. mcintoshi* and *Ae. dentatus*, representing 51 and 46%, respectively, of the total *Aedes* species (Linthicum et al. 1988). Selected rearings during the present study produced all the above species except *Ae. unidentatus* and rearings during the fourth flooding yielded *Ae. mcintoshi*, *Ae. dentatus* and *Ae. circumluteolus* from both sites. Species identification of *Aedes* eggs was not possible and thus could not be used to determine the relative abundance.

In the present study, significantly more floodwater *Aedes* eggs hatched during the first flooding in this study than during subsequent floodings. This is in contrast to the rock-pool breeding of *Ae. vittatus* in which few eggs hatched during the first soaking (Service 1970) or the salt marsh breeding *Ae. detritus* that yielded less than 5% hatch during the first soaking (Service 1968). The egg-hatch characteristic of the floodwater *Aedes* spp. located in the dambos (i.e., >90% hatch during the first flooding) may provide an opportunity for control measures to be applied effectively against the immature stages. One application of a sustained-release formulation of methoprene in Altosid® pellets was completely effective in blocking both *Aedes* and *Culex* adult emergence for at least 2 wk after flooding in a similar dambo system (Linthicum et al. 1989). The same formulation can be used as a pretreatment in mosquito-breeding areas several weeks in advance of flooding with similar effectiveness, allowing the applicator to treat these flood-prone areas before access roads become impassable due to the onset of rains. Control of the initial hatch of *Aedes* spp. in RVF endemic areas may reduce the population of transovarially infected vectors to a level low enough to prevent the initiation of RVF epizootics.

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