IMPACT OF STRESSFUL CONDITIONS ON THE SURVIVAL OF *CULEX PIPIENS* EXPOSED TO RIFT VALLEY FEVER VIRUS[1]

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ABSTRACT. Several groups of Rift Valley fever (RVF) virus-exposed and unexposed *Culex pipiens* were allowed differential access to a carbohydrate food source and their survival monitored. When stressed by deprivation of a carbohydrate source, mean survival times of RVF virus-exposed mosquitoes were consistently higher than those of unexposed mosquitoes in each of the carbohydrate-deficient experiments. These differences were statistically significant when mosquitoes were provided 5% sucrose for 24 hours. Mosquitoes that were provided access to a carbohydrate source for 24 h after a bloodmeal and then were denied access survived significantly longer than did those mosquitoes denied access, regardless of their exposure to RVF virus. When not stressed, RVF virus-exposed individuals had slightly higher daily survival rates than did unexposed individuals.

INTRODUCTION

Culex pipiens (Linn.) has been incriminated as a potential vector in the epizootic cycle of Rift Valley fever (RVF) virus in Africa, based upon virus isolations from field-collected specimens (Meegan et al. 1980, Linthicum et al. 1985b) and demonstrated vector competence in the laboratory (Meegan et al. 1980, Gargan et al. 1983, Turell et al. 1984). This species was implicated as the principal vector during an epizootic occurring in Egypt in 1977 (Hoogstraal et al. 1979) and was likely involved in the epidemic/epizootic in Mauritania in 1987 (Linthicum et al. 1990). Under optimal laboratory conditions, female *Cx. pipiens* with a disseminated RVF virus infection have reduced fecundity, survival and ability to refeed when compared with uninfected specimens (Turell et al. 1985, Faran et al. 1987). Adverse environmental conditions observed in the Sahel ecosystem in Mauritania during the 1987 epizootic/epidemic suggest that vectors of RVF virus may have been stressed by environmental conditions. To investigate the possible interaction of environmental stress and RVF virus infection on the survival of mosquitoes, several groups of *Cx. pipiens* were allowed to feed on either viremic or nonviremic hamsters, given differential access to a carbohydrate source, and their survival monitored.

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MATERIALS AND METHODS

Syrian hamsters (*Mesocricetus auratus*) were inoculated intraperitoneally with 10^4 plaque-forming units (PFU) of the ZH501 strain of RVF virus (Meegan 1979) approximately 24 h before mosquito exposure. *Culex pipiens* (El Gabal strain) from F_{120} to F_{132} generations were reared uniformly under rigorously controlled conditions (Gargan et al. 1983). Each trial included mosquitoes from a single generation only. Four- to 6-day-old female mosquitoes were divided into 2 randomly selected groups and allowed to feed either on an anesthetized viremic or a nonviremic hamster (3 replicates). After each feeding, equal numbers (approximately 50) of engorged mosquitoes were placed in 3.8-liter cardboard containers with netting at one end. Mosquitoes in these paired cages (exposed, unexposed) were allowed one of 4 feeding regimens. These consisted of: 1) 5% sucrose solution for 24 h, followed by distilled water only (6 replicates); 2) 20% sucrose solution for 24 h, followed by distilled water only (3 replicates); 3) distilled water only throughout the experiment (2 replicates); and 4) 5% sucrose solution throughout the experiment (3 replicates). Containers were placed in a plastic bag with a moist sponge to maintain high relative humidity. Temperature was held at 26°C under a 16L:8D photoperiod. Survival was monitored and dead mosquitoes were removed and counted daily. A Student's t-test ($\alpha = 0.05$) was used to compare the mean number of days that mosquitoes that fed on a viremic host survived with those that fed on a nonviremic host for each regimen. Upon death, each mosquito was triturated in 1 ml of diluent (10% heat-inactivated fetal bovine serum in Medium 199 with Hanks' salts, $NaHCO_3$ and antibiotics) and tested for virus by plaque assay on 2- to 4-day-old Vero cell monolayers (Gargan et al. 1983). Mean virus titers in these mosquitoes ranged from 10^5 PFU (day 1) to 10^3 PFU (day 15). Only those specimens from which virus was recovered (455/688, 66%) were assigned to the "fed on viremic host" category. An anthrone test (Van Handel 1972) was conducted on blood-fed mosquitoes, half of which were offered sucrose, and the remainder, water only, for 24 h after ingestion of a bloodmeal. Research was conducted in compliance with the Animal Welfare Act and other Federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the *Guide for the Care and Use of Laboratory Animals*, NIH publication 86-23, 1985 edition.

RESULTS

There was no significant difference in survival within any regimen; therefore, the data within each regimen were combined for further analysis. In regimens 1–3, deprivation of a carbohydrate source resulted in high mortality rates 2–3 days after the carbohydrate source was removed. For each regimen, virus-exposed mosquitoes survived longer than did unexposed mosquitoes (Table 1). This difference was significant ($P < 0.01$) for those mosquitoes receiving 5% sucrose for 24 h, but not for those in the other regimens. Also, mosquitoes that were denied sucrose died sooner ($P < 0.001$) than did those that were provided sucrose for 24 h and then starved (Table 1). The anthrone test confirmed that mosquitoes ingest sucrose within 24 h of a replete blood meal. All 10 (100%) engorged mosquitoes provided 20% sucrose for 24 h had a positive anthrone test. In contrast, none (0%) of 15 engorged mosquitoes provided water only had a positive test 24 h after taking a bloodmeal.

Table 1. Survival of adult *Culex pipiens* fed on a Rift Valley fever viremic hamster or a nonviremic hamster, and exposed to various regimens of sucrose as a carbohydrate source.

Carbohydrate source for first 24 h[a]	Exposed to	
	Viremic host	Nonviremic host
20% sucrose	5.1 ± 0.2 (50)[b]	4.7 ± 0.2 (91)
5% sucrose	5.1 ± 0.1 (183)	4.5 ± 0.1 (289)
Water only	4.0 ± 0.1 (79)	3.9 ± 0.1 (125)
5% sucrose throughout	98.6%[c] (100–97.2)	96.5% (98.6–94.4)

[a] Mosquitoes were provided the indicated concentration of sucrose for 24 h after a replete bloodmeal and only water thereafter.

[b] Mean number of days survived ± SE (no. tested).

[c] Daily survival rate (95% confidence), calculated from the antilog of the regression coefficient, was used to quantitate survival because the mean number of days survived could not be calculated for this group due to their extended survival (>15 days).

Mosquitoes survived significantly better when sucrose was provided throughout the experiment (regimen 4). Therefore, daily survival rates were estimated by regression of linearized survival curves. Regression lines were determined by using the Statgraphics Simple Regression Procedure (STSC Plus*Ware 1986). Daily survival rates were defined as the antilog of the slope of the regression line (antilog of the regression coefficient) (Linthicum et al. 1985a). Mosquitoes that fed on viremic or nonviremic hosts had similar survival rates, 99% for those fed on viremic hamsters, and 97% for those fed on nonviremic hosts (Table 1). Survival rates for the mosquitoes that fed on viremic hosts were, however, slightly higher than survival rates for mosquitoes that fed on nonviremic hamsters.

DISCUSSION

Despite previous studies that indicated increased mortality rates, decreased fecundity and reduced refeeding in *Cx. pipiens* with disseminated RVF virus infections when held under optimum laboratory conditions (Turell et al. 1985, Faran et al. 1987), we did not observe increased mortality rates in virus-exposed mosquitoes. In fact, the virus-exposed mosquitoes survived 0.6 (±0.1) days longer than unexposed mosquitoes when both were fed 5% sucrose for 24 h after a bloodmeal. This apparent conflict may be because Faran et al. (1987) compared mosquitoes with disseminated infections with those without a disseminated infection (including both uninfected mosquitoes and those that were infected, but without a disseminated infection). Since about 25% of *Cx. pipiens* develop a disseminated infection after oral exposure (Turell et al. 1984), it is likely that the majority of mosquitoes in our study had nondisseminated infections, and thus were not at risk of higher mortality.

Environmental conditions play a significant role in the survival of mosquitoes (Nayar and Pierce 1980, Kaul et al. 1984, Gad et al. 1989). We intended the deprivation of sucrose (i.e., a carbohydrate source) to simulate environmental conditions similar to those observed in Mauritania during the 1987 epizootic in which there were large populations of *Cx. pipiens* and other potential vectors (Linthicum et al. 1990) and little or no vegetation available to serve as a carbohydrate source. Stressful environmental conditions may exacerbate the effect of RVF virus infection on survival of mosquitoes. However, when starved, RVF virus-exposed mosquitoes survived better than those not exposed to this virus. Virus infections might behaviorally and physiologically affect vectors, making them more or less susceptible to environmental conditions. For instance, infection may lead to vector inactivity. This could delay the effects of starvation. In a field situation, this may also reduce exposure to predators. Another possible explanation may be that infection in a vertebrate host may affect nutrients available for vectors in the host blood. Increased survival among infected mosquitoes may afford greater opportunity to transmit to a secondary host.

Although nectar contains a complex mixture of sugars and other organic compounds, sucrose is the dominant sugar in nectars of many plants, and nectar can contain up to 50% sugar (Free 1970). While we did not duplicate natural conditions exactly, we would argue that the intake of assimilable sugars ultimately represents input to the overall caloric reserves of a mosquito, and that it is these caloric reserves that are directly relevant to the influence of virus infection on mosquito survival. Previous studies (Foster 1986, Foster et al. 1986) indicate that mosquitoes may ingest nectar within 24 h of a replete bloodmeal. The question remained, would the energy in this nectar meal, in addition to that already present in the bloodmeal, have any effect on mosquito survival during subsequent periods when additional carbohydrates were not available. Our study indicated that ingestion of sucrose by *Cx. pipiens* within 24 h of a replete bloodmeal enhanced mosquito survival, as compared with that of mosquitoes not provided sucrose during the same time period, when a carbohydrate source became unavailable.

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