DETECTION OF ST. LOUIS ENCEPHALITIS VIRUS IN MOSQUITOES BY USE OF THE POLYMERASE CHAIN REACTION

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ABSTRACT. We recently developed an assay using the polymerase chain reaction (PCR) for the specific detection of St. Louis encephalitis (SLE) virus RNA. This assay was tested in a blind study on 7 samples of pooled mosquitoes (50 mosquitoes/pool) which were also characterized for SLE virus by plaque assay in Vero cell culture. One sample was positive for the SLE virus as determined by both the PCR assay and a combination of the plaque assay and the indirect fluorescent antibody assay. The remaining 6 samples were negative for the presence of SLE virus as determined by both methods. These data indicate that this PCR assay can be used to monitor for the presence of SLE virus in pools of homogenized mosquitoes. This approach could provide early data on which to base disease control decisions.

St. Louis encephalitis (SLE) virus is a major mosquito-borne pathogen which causes periodic disease outbreaks throughout North, Central, and South America (Monath 1990). Control of SLE outbreaks currently depends on monitoring for SLE virus activity in the natural vertebrate hosts and vectors (i.e., birds and mosquitoes) and using mosquito abatement when the virus is detected. The presence of SLE virus has typically been detected by assaying for antibodies to SLE virus in blood drawn from sentinel chickens or wild birds or by isolation and growth of the virus from vertebrate hosts and mosquitoes through intracranial inoculation of suckling mice, infection of cell cultures, or enzyme immunoassay (Tsai et al. 1987, 1988). In various ways, these techniques have had problems with reliability or are laborious and time-consuming.

The polymerase chain reaction (PCR) is a relatively new technique which has been used to specifically and sensitively detect a variety of infectious pathogens. We recently developed a PCR-based assay for the specific detection of SLE virus RNA (Howe et al. 1992). This assay was able to detect viral RNA spiked into a background of homogenized mosquito abdomen. Others have used the PCR for detecting the RNA of SLE virus and additional flaviviruses (Eldadah et al. 1991). The likely use for these assays is in the detection of virus RNA in mosquitoes since viremia levels in patients typically disappear before clinical symptoms appear (Calisher and Monath 1988). In the current study, we have utilized additional processing methods and tested our PCR-based assay in a blind study on pooled samples of mosquitoes collected in August 1991 near Pine Bluff, Arkansas during an SLE outbreak in that region (Savage et al., unpublished data).

Mosquitoes were collected by using either a CDC light trap with dry ice or a CDC gravid trap (Reiter 1987). Adult mosquitoes were identified and pooled by species or taxonomic group using dissecting microscopes on chill tables kept at about 4°C. Mosquito pools (50 mosquitoes per pool) were triturated in 2 ml of BA-1 diluent (0.2 M Tris, pH 8.0, 0.15 M NaCl, 1% BSA, 10 mg/l phenol red, 50 μg/ml gentamicin[5] and one μg/ml Fungizone[5]) using cold mortars and pestles. Suspensions were centrifuged at 14,000 rpm for 2 min. Supernatants were poured into 1-dram screw-cap vials and stored at −70°C. The suspensions were tested for virus by plaque assay in Vero cell culture as previously described by Mitchell et al. (1987).

To assay the samples by the PCR, total RNA was extracted from 100-μl aliquots of the mosquito suspensions by using the RNaid Plus kit (Bio 101, La Jolla, CA) according to the manufacturer's instructions. Briefly, the samples were lysed with guanidine isothiocyanate and extracted with acid-phenol. The RNA was bound to a powdered-glass matrix, washed and eluted from the matrix in diethyl pyrocarbonate (DEPC)-treated water. The RNA was eluted in 20 μl (1/5 the samples original volume, or 5× the samples original concentration) of water, and this 5×-concentrated RNA suspension was then diluted 10-fold to 0.5× concentration. Two-μl aliquots of the RNA suspensions were assayed by reverse transcription/PCR using the amplification primers SLE364.for (5′-GATCCATG-

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Table 1. Collections of pooled mosquitoes from Pine Bluff, Arkansas. Results of plaque assay in Vero cell culture and the PCR assay.

Pool number	Species	Plaque Assay	PCR Assay
AR91-3105	*Anopheles quadrimaculatus*	–	–
AR91-2993	*An. quadrimaculatus*	–	–
AR91-3026	*An. quadrimaculatus*	–	–
AR91-3031	*Culex pipiens* complex	–	–
AR91-3074	*Cx. pipiens* complex	+	+
AR91-3133	*An. quadrimaculatus*	–	–
AR91-3115	*Culex erraticus*	–	–

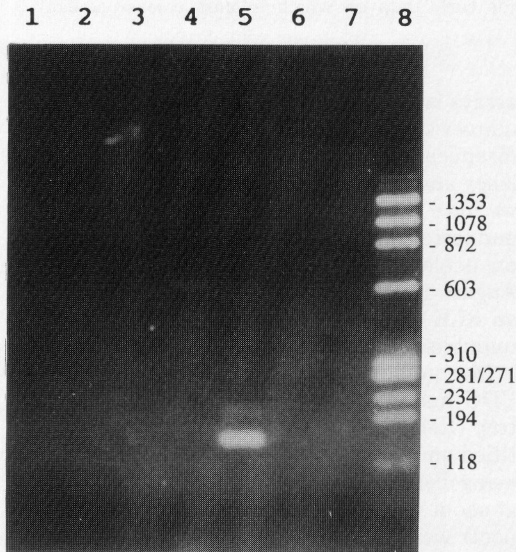

Fig. 1. Agarose gel electrophoresis of amplification products from samples of Arkansas mosquitoes. The RNAs from RNaid-extracted mosquito samples were reverse-transcribed, and the cDNAs were amplified by the PCR using SLE364.for and SLE525.rev primers (expected product=161 bp) in 50-μl reaction volumes. Ten-μl aliquots of the reactions were electrophoresed in a 2% agarose gel which was then stained with ethidium bromide and visualized under ultraviolet light. Lane 1, AR91-3105; lane 2, AR91-2993; lane 3, AR91-3026; lane 4, AR91-3031; lane 5, AR91-3074; lane 6, AR91-3133; lane 7, AR91-3115; lane 8, ϕX 174-Hae III molecular weight marker, with numbers on right indicating the size in base pairs.

CTAGACACCA-3′) and SLE525.rev (5′-GCG-CTTTGAGCGTCAGT-3′) as described previously by Howe et al. (1992).

The results of the plaque assay and the PCR assay are listed in Table 1. One sample, AR91-3074, was positive for SLE virus as determined by the plaque assay. The virus titer of this sample was determined to be 56 plaque-forming units (PFU) per 0.1 ml of sample. Subsequent analysis of sample AR91-3074 using an indirect fluorescent antibody (IFA) test identified both the SLE virus and an additional virus, Flanders (FLA) virus or one closely related to it (Nick Karabatsos, personal communication). It is not known what proportion of the PFUs in sample AR91-3074 are SLE virus and what proportion are a FLA-like virus. The remaining 6 samples were negative for SLE virus as determined by plaque assay. The PCR assay also gave a positive result for SLE virus in sample AR91-3074 as indicated by amplification of the predicted 161-base pair (bp) DNA fragment (Fig. 1). The remaining 6 samples were negative for SLE virus as determined by the PCR assay.

The PCR assay detected SLE viral RNA from an aliquot of purified nucleic acid which represented 1 μl of the original mosquito suspension. The plaque assay determined the virus titer to be 0.56 PFU/μl. This suggests that the PCR assay detected SLE viral RNA in an aliquot which contained less than 0.5 PFU of SLE virus.

It is unlikely that infectious viral particles provide the only RNA template for reverse transcription and amplification. A large proportion of the viral RNA template probably consists of unpackaged viral RNAs or RNA in defective viral particles. This may also imply that the PCR assay could detect the presence of SLE viral RNA in mosquitoes before they are packaged and become infectious. The PCR assay may provide a more sensitive and an earlier indicator of the presence of SLE virus in comparison to assays which rely on detecting infectious particles of SLE virus in mosquitoes or antibodies to SLE virus in avian hosts.

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