Chikungunya fever is an arthropod-borne disease caused by chikungunya virus (CHIKV) belonging to genus Alphavirus, family Togaviridae. CHIKV has re-emerged in India, Indian Ocean Islands, and South East Asia in an explosive outbreak affecting ~6 million people in 2005–06. During the resurgence, the virus caused severe morbidity and clinical complications that were hitherto unknown of CHIKV. Neurological, ophthalmological, renal, and cutaneous involvement was more evident during the recent outbreak in addition to encephalitis/encephalopathy. The CHIKV-associated mortality was very high in La Reunion Island and India and accounted for several thousand deaths. The virus is transmitted to humans by the bite of infected *Aedes aegypti* mosquitoes, the principal vector of chikungunya in urban areas and *Ae. albopictus* mosquitoes in peri-urban areas. The recent mutation in the CHIKV genome has helped the virus to adapt to the new vector, *Aedes albopictus* for rapid dissemination and transmission. Vector control is the only means to break virus transmission as no effective therapies or vaccines are in place for the virus. Proactive surveillance of vector species for detection of CHIKV is an accepted mode of surveillance where wild-caught mosquitoes are screened routinely as part of the virus detection program. However, a major concern was the maintenance of a cold chain during transport of mosquitoes from field to laboratory in many tropical countries. The recent report of dengue virus detection in dried *Ae. aegypti* mosquitoes by reverse transcriptase-polymerase chain reaction (RT-PCR) have prompted us to take up a study in the same direction. In this study, we report the methodology of mosquito storage and the persistence of CHIKV RNA in experimentally infected mosquitoes after prolonged storage at 28°C.

**Abstract.** Experiments were conducted to determine the persistence of chikungunya viral (CHIKV) RNA in experimentally infected *Aedes aegypti* mosquitoes stored for prolonged periods at 28°C. Intra-thoracically inoculated mosquitoes with confirmed positivity were killed by quick freezing at −80°C, applied to sticky tape, and stored at 28°C with 80 ± 5% relative humidity (RH). At weekly intervals, five mosquitoes were removed from the tape randomly and assayed individually for detection of viral RNA by reverse transcriptase-polymerase chain reaction (RT-PCR). CHIKV RNA was detected up to 12 weeks in dry mosquitoes by RT-PCR. Virus could not be isolated either in cell culture or in the suckling Swiss-albino mouse system at any stage. This study demonstrated the persistence of CHIKV viral RNA up to 12 weeks when stored at 28°C with RH 80 ± 5%. This finding will have significance in CHIKV surveillance programs in mosquito populations or field-based studies in countries where maintenance of a cold chain is a concern.
12 weeks (Figure 1). The band intensity of samples up to Week 11 was high, whereas a faint band appeared for the Week 12 sample. No band was obtained for Week 13 and Week 14 samples, and this could be caused by either complete degradation of RNA or the amount of RNA below the limit of detection. The study has clearly demonstrated CHIKV RNA persistence up to a maximum of 12 weeks under the above conditions in mosquitoes. All the mock-infected samples tested negative up to a maximum of 12 weeks under the above conditions in mosquitoes. The technique has been successfully applied in several studies to screen mosquito suspensions using suckling mice (Swiss albino) and tissue culture were futile despite inoculating the suspensions for two consecutive passages.

The sticky tape method for transport of mosquitoes from CHIKV-endemic areas for CHIKV RNA detection in the laboratory using RT-PCR would play an important role in the surveillance of CHIKV in field-collected mosquitoes. The technique has been successfully applied in several studies to screen mosquitoes for arboviruses. Bangs and coworkers demonstrated the persistence of dengue viral RNA in dried mosquitoes up to 30 days after application to the lure. Subsequent studies by the authors demonstrated the persistence of dengue virus RNA in dried Aedes aegypti mosquitoes for 13 weeks under tropical conditions. The persistence of Western equine encephalomyelitis and St. Louis encephalitis viral RNAs in mosquitoes up to 20 days in the absence of a cold chain has also been reported. Similar results were also reported for West Nile and Japanese encephalitis viruses stored at room temperature for 14 days. Guzman and colleagues demonstrated the persistence of Venezuelan equine encephalitis virus RNA for 40 days. This study has demonstrated the persistence of CHIKV RNA up to 12 weeks in mosquitoes stored at 28°C with high humidity (80 ± 5%).

The RT-PCR has been a reliable tool for specific and rapid detection of CHIK viral RNA in serum and mosquito samples. The technique has been used successfully for rapid diagnosis of many viral agents during outbreaks. The applicability of the technique in this study to detect CHIKV RNA in dry mosquitoes up to 12 weeks shows its efficacy. The persistence of CHIKV RNA at 28°C with 80 ± 5 RH% up to 12 weeks in dry mosquitoes on sticky traps is a significant observation and has considerable epidemiological importance. Though CHIKV is comparatively stable at room temperature in serum samples, it is not clear how the viral RNA is preserved in dead mosquitoes for prolonged periods. However, this study clearly shows that maintaining of cold chain can be omitted when RT-PCR is used as the diagnostic technique for RNA detection. This technique will most appropriately fit during field-based virus surveillance in mosquitoes. The technique will have significant application, especially in third world countries where limited resources are available to carry out virus surveillance in mosquitoes and other virus vectors.

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