RECOVERABILITY OF RIFT VALLEY FEVER AND SANDFLY FEVER SICILIAN VIRUSES FROM INFECTED *PHLEBOTOMUS PAPATASI* (DIPTERA: PSYCHODIDAE) TRAPPED IN VARIOUS OILS

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ABSTRACT. We studied the effects of various oils used to trap sand flies on the recovery of virus from infected adult *Phlebotomus papatasi*. Both Rift Valley fever and sandfly fever Sicilian viruses were readily recovered from virus-inoculated specimens held at 26°C on mineral, olive or castor oil-soaked sheets for up to 12 h. However, after 50 h on oil-soaked paper, significantly greater titers were recovered from sand flies trapped with mineral oil than from sand flies trapped with either of the other oils. This indicates that sand flies trapped on oil-soaked paper would be suitable for virus isolation attempts and that mineral oil had the least effect on virus recovery.

Phlebotomine sand flies have been incriminated as vectors of numerous disease agents, including viruses, protozoa and bacteria (Gubler 1984). Various methods (e.g., light traps, sticky traps, aspiration, etc.) have been used to capture sand flies to determine field-population densities and species distributions as well as to monitor for the prevalence of disease producing organisms (Chaniotis 1978). However, using light traps may not always be possible, such as during periods of military activity, when these traps may give information about troop activity. Oil-soaked papers placed in areas frequented by sand flies have been shown to be an effective trap for these flies (Lindquist 1936, Asimeng 1988, Perkins et al. 1988). Although viruses have been recovered from sand flies trapped on oil-soaked surfaces (Aitken et al. 1975), the potential impact of this trapping method on virus isolation attempts has not been evaluated.

To determine if trapping sand flies on oil-soaked paper affected virus isolation from these specimens, we studied the effect of various sand fly-trapping oils on the recovery of Rift Valley fever (RVF) and sand fly fever Sicilian (SFS) viruses from adult *Phlebotomus papatasi* (Scopoli). *Phlebotomus papatasi* is a natural vector of SFS virus (Tesh 1988) and is able to transmit RVF virus in the laboratory (Turell, unpublished data).

Sand flies, *P. papatasi* (ISRAEL strain), used in this study came from a colony maintained at the Walter Reed Army Institute of Research, Washington, DC, and originated from a colony at the Hadassah Medical School, Jerusalem, Israel. The number of generations in colonization is not known. Sand flies were reared by the methods of Endris et al. (1982) using the larval food described by Young et al. (1981).

The ZH501 strain of RVF virus, isolated from a 10-year-old girl with a fatal RVF infection in Egypt in 1977 (Meegan 1979), was used after 2 passages in fetal rhesus lung cells. The prototype strain of SFS, isolated from human serum, had been through 37 passages in suckling mouse brain, 6 in Vero cells, and 2 in Vero E6 cells. Each sand fly was inoculated intrathoracically (Rosen and Gubler 1974) with about $10^{2.5}$ plaque-forming units (PFU) of either RVF or SFS virus in a volume of 0.1 μl of suspension. The inoculated sand flies were placed in 0.9-liter cardboard cages with fine netting at one end and provided diluted Karo corn syrup (1:1 in water) as a carbohydrate source. The cages were placed in plastic bags along with a moist sponge to maintain a high humidity and held in an incubator at 26°C.

After 4 days, dead sand flies were removed and the remaining flies were cold anesthetized. The anesthetized sand flies were dropped from a height of about 20 cm onto sheets of paper (22 × 14 cm) soaked in mineral, castor or olive oil. Approximately 25% of the anesthetized sand flies were placed back into the cardboard holding cage. These flies all recovered from anesthesia, indicating that the cold anesthesia did not kill the flies, and were assayed at selected times to serve as positive controls. In addition to the oil-trapped flies held at 26°C, a second set of flies, trapped on a castor oil- or a mineral oil (SFS virus only)-soaked piece of paper, were placed at 30°C. A sample of 4 or 5 flies was obtained from each sheet of paper at 12, 24 and 50 h after they had been placed on the paper. At each sampling time, 3 to 5 flies were removed from the cardboard holding cage and tested as positive controls. Flies were triturated individually in 1 ml of diluent (10% fetal bovine serum in Medium 199 with Hanks' salts, antibiotics, and $NaHCO_3$) and frozen at −70°C until tested for infectious virus by plaque assay on Vero cell monolayers (Gargan et al. 1983).

Although all sand flies trapped on oil-soaked paper were dead when checked at 12 h, both RVF and SFS viruses were readily detected from flies sampled at 12 h with all oils tested (Figs. 1

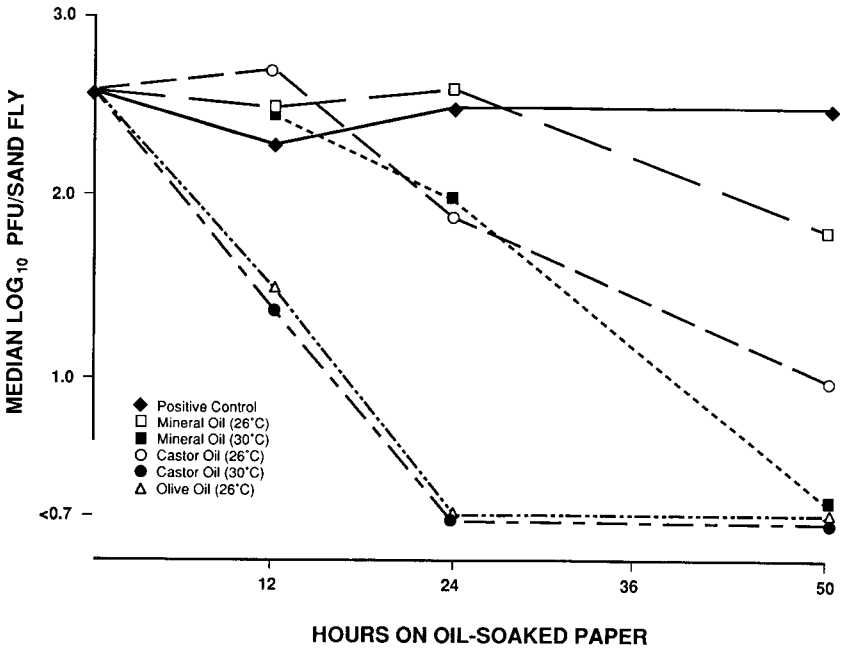

Fig. 1. Median $\log_{10}$ PFU of SFS virus recovered from *Phlebovirus papatasi* is shown for live controls or sand flies trapped for 12, 24 or 50 h on oil-soaked paper. Five sand flies were sampled at each point.

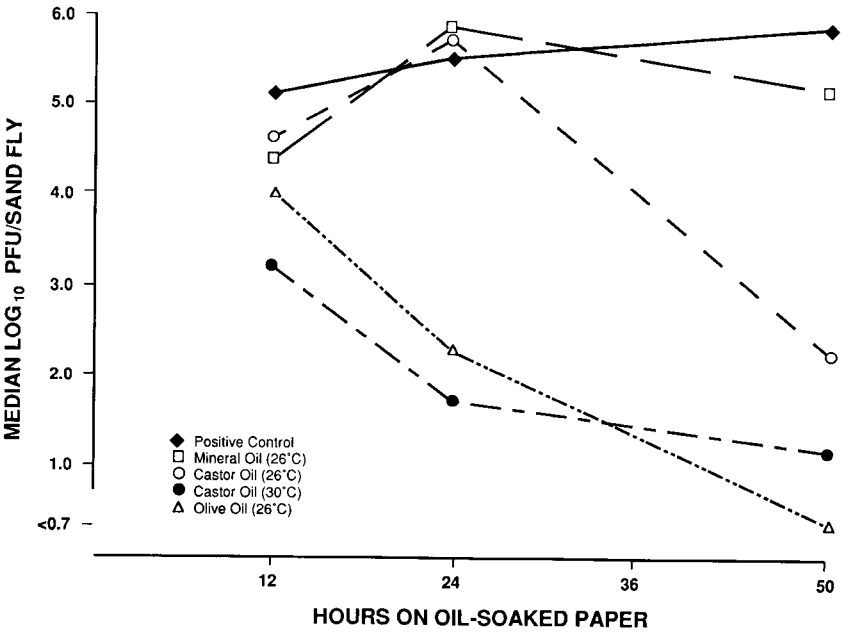

Fig. 2. Median $\log_{10}$ PFU of RVF virus recovered from *Phlebovirus papatasi* is shown for live controls or sand flies trapped for 12, 24 or 50 h on oil-soaked paper. Between 3 and 5 sand flies were sampled at each point.

and 2). However, by 24 h at 26°C, significantly (t-test, $P < 0.05$) less virus was recovered from flies trapped on the olive oil-soaked paper than from flies trapped on either the mineral or castor oil-soaked papers or the positive controls. Virus titers recovered from flies trapped in either mineral or castor oil did not differ statistically from those of positive controls. Likewise, virus titers in flies trapped for 50 h at 26°C on mineral oil-soaked paper were not statistically different from titers in the positive control flies. Virus titers decreased significantly (t-test, $P < 0.005$) in flies trapped for 50 h on castor oil-soaked paper with both viruses.

At 30°C, titers with both viruses decreased more rapidly than when flies were held at 26°C. We were unable to detect SFS virus from flies trapped with mineral oil for 50 h or from castor oil-trapped flies after 24 h (Fig. 1). Similar results were observed with sand flies inoculated with RVF virus and trapped on castor oil-soaked paper at 30°C (Fig. 2).

To determine if any of the oils had a direct effect on virus recovery, we added RVF virus to diluent or to diluent containing 0.1 or 1% concentrations of each of the 3 oils. A sample of each of these suspensions was obtained immediately after the addition of virus and again after 24 and 48 h at ambient room temperature. These samples were frozen at −70°C until tested for virus as described above. Titers of virus recovered at each sampling point from each of the suspensions were nearly identical (data not shown). Thus, the presence of oil itself did not appear to affect virus recovery, and the differences in virus recovery among flies trapped in the various oils in our study may be due to an interaction between the oil and sand fly tissue on virus stability (i.e., flies trapped in olive oil may decompose faster than those trapped in mineral oil).

We have demonstrated that sand flies, trapped on papers soaked in any of the oils tested, would be suitable for virus isolation if flies are processed within 12 h. If a longer collection period is desired, then mineral oil would be the better choice. Oil selection is further complicated by anecdotal reports, which indicate that both olive and castor oils are attractive to sand flies, thus enhancing trapping efficacy. Therefore, depending on environmental temperature, processing frequency of trapped flies and the desired population size will dictate the choice of oil.

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