Failure to Isolate Viruses of the Genus *Phlebovirus* from *Lutzomyia shannoni* Sand Flies (Diptera: Psychodidae) from Ossabaw Island, Georgia[1]

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ABSTRACT *Lutzomyia shannoni* Dyar were collected on Ossabaw Island, Chatham County, GA, and tested for infection with viruses of the genus *Phlebovirus*. No virus isolates were obtained from 5,053 sand flies collected in 1990 and 1991. These results suggest that phleboviruses are absent from this population of sand flies.

KEY WORDS Diptera, sand fly, *Lutzomyia shannoni, Phlebovirus*, virus.

The genus *Phlebovirus* (Bunyaviridae) contains 38 distinct virus serotypes which occur in southern Europe, Africa, central Asia, and in the Americas. Although a few phleboviruses occur in temperate areas, most of the viruses are found in tropical and subtropical regions. Eight of the viruses have been associated with disease in humans (phlebotomus fever group viruses), and one (Rift Valley fever virus) is an important pathogen of domestic animals in Africa (Tesh 1988).

The isolation of Rio Grande (RG) virus from pack rats (*Neotoma micropus*) in south Texas was the first demonstration of a naturally-occurring phlebovirus in the United States (Calisher et al. 1977). Subsequently, replication and transovarial transmission of RG virus were demonstrated in *Lutzomyia anthophora,* a phlebotomine sand fly that lives in close association with pack rats and is the suspected vector of RG virus (Endris et al. 1983). These findings, coupled with the detection of RG virus-neutralizing antibodies in a variety of small mammals in south Texas, including pack rats, suggested the presence of a stable focus of RG virus activity in that area.

Recent studies with *L. shannoni* have implicated this species as a vector of vesicular stomatitis virus (VSV) in Georgia (Comer et al. 1990, 1991). The possibility was raised that this species might be infected with other arboviruses at this site. The apparent focus of RG virus in south Texas, coupled with the dearth of information on the possible presence of this or other phleboviruses elsewhere in the United States, led to the testing of *L. shannoni* collected in Georgia for infection with any of these agents.

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Materials and Methods

Sand flies were collected on Ossabaw Island, Chatham County, GA, as part of a long-term study of the epizootiology of VSV being conducted at this site. A funnel trap was used to capture the flies as they emerged from hollow trees, the primary diurnal resting shelter for this species at this locale (Comer and Corn 1991). Sand flies were pooled, triturated in 2.0 ml of diluent, and tested for VSV infection as described previously (Comer et al. 1992). Pools were then stored at -80°C for further testing for phleboviruses.

Pools that contained large numbers of sand flies and which were negative for VSV infection were thawed and prepared for phlebovirus testing. The protocol varied slightly between years; in 1990, new pools derived from 0.1 ml aliquots from 5 to 19 VSV-negative pools, whereas in 1991, 0.2 ml aliquots from 4 to 6 VSV-negative pools were so combined. The resulting pools were inoculated onto monolayers of Vero cells which were grown in 75 cm^2 flasks maintained at 37°C. Following adsorption for 1 hour, each flask received 25 ml of maintenance media and was returned to the incubator. Flasks were examined daily for 14 days. Maintenance media were changed twice during this period. If no cytopathic effects were observed in the Vero cell monolayer during the 2-week interval, the assay for virus was considered negative.

Results and Discussion

A total of 5,053 sand flies were assayed for virus in 27 pools (Table 1). No viruses were isolated. The majority of the phleboviruses are associated with sand flies belonging to the genera *Phlebotomus* or *Lutzomyia* (Tesh 1988), and all of the serotypes presently known produce cytopathic effects in Vero cells cultured in liquid medium (Tesh 1989). Therefore, it seemed reasonable to test *L. shannoni* for infection with one of these agents, and Vero cell culture was the assay system of choice.

The frequency with which phleboviruses have been encountered in sand flies collected in diverse regions of the world, including Brazil (1 phlebovirus isolate per 602 sand flies tested, Aitken et al. 1975), Panama (1 per 2,275 flies, Tesh et al. 1974), Iran (1 per 208 flies, Tesh et al. 1977), and Italy (1 per 195 flies, Verani et al. 1988), together with the numbers of flies tested here, suggest that phleboviruses were not present in the sand fly population on Ossabaw Island, or if so, that the prevalence of infection in *L. shannoni* was too low to detect, given the numbers of specimens tested. Although 83% of the sand flies tested were males, which do not feed on blood, 9 of the 38 phlebovirus serotypes presently known have been isolated from naturally (and presumedly transovarially) infected male sand flies (Tesh 1989); therefore the testing of this sex, which predonderates in field collections (Comer and Corn 1991), was appropriate. No additional testing for phleboviruses is planned.

Table 1. Number of *Lutzomyia shannoni* collected on Ossabaw Island, Chatham County, GA, in 1990 and 1991 and which tested negative for *Phlebovirus* infection.

Year	Males		Females	
	Individuals	Pools	Individuals	Pools
1990	3,087	13	496	7*
1991	1,105	3	365	4

* Six of these pools contained a total of 37 females with partially digested blood.

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