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SURVIVAL OF ARBOVIRUSES IN TRYPANOSOME-INFECTED TRIATOMINES*

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Abstract. The potential of triatomines to maintain arboviruses was demonstrated by the ability of *Rhodnius prolixus* with experimentally punctured abdomen to harbor Venezuelan equine encephalitis (VEE) virus for at least 4 months and St. Louis encephalitis virus for 1 month. At 30 days after infection VEE virus was found at low titers in *Trypanosoma cruzi*-infected *R. prolixus* and at moderate titers in *T. rangeli*-infected *R. neglectus*. Transmission of VEE virus by bite of punctured bugs was successful 2 weeks after virus ingestion; attempts at 30 days failed.

Despite efforts to incriminate triatomines, commonly known as assassin bugs (Hemiptera: Reduviidae), as vectors of arboviruses, their role in arbovirus ecology is still unclear. Kitselman and Grundmann reported the isolation of western equine encephalitis (WEE) virus from wild-caught *Triatoma sanguisuga*.[1] More recent investigations by Mangiafico et al.[2] in which WEE was maintained for up to 98 days in *Rhodnius prolixus* which had been punctured experimentally stimulated our studies of the role of triatomines in sustaining natural transmission cycles of arboviruses.

The infection of triatomines with trypanosomes may be an important factor in the bugs' susceptibility to arbovirus infection. *Trypanosoma rangeli* penetrates the digestive tube wall, enters the hemocoel and migrates to the salivary glands.[3-5] Such a mechanism may simulate the artificial process of puncturing triatomines.

The purpose of the present study was to assess the vector potential of normal, punctured, and parasitized triatomines.

MATERIALS AND METHODS

Viruses

Venezuelan equine encephalitis (VEE), MF-8 epizootic strain, was isolated in 1969 from human blood in Central America.[6] This virus was passaged once in suckling mice by the intracerebral (ic) route and four times in Vero cells before inoculating 1- to 2-day-old mice with 1,000 to 10,000 plaque forming units (pfu) by the intraperitoneal (ip) route. The animals developed a viremia reaching approximately 7 log pfu/ml of blood 18 to 26 hours after injection, at which time they were used as a source of infective blood meals for bugs.

St. Louis encephalitis (SLE) virus was isolated in Panama from an Olivaceous Cormorant (*Phalacrocorax olivaceus*) in 1973. The first Vero passage level was used to infect 1- to 2-day-old mice as above, producing a viremia of approximately 6 log pfu/ml of blood within 48 to 96 hours after inoculation.

Triatomines

Rhodnius prolixus, *R. neglectus*, and *Panstrongylus herreri* colonized in this laboratory since 1965 were used in the experiments. They were held individually in upright plastic 10-dram vials with filter paper on the bottom. The mouth of the vials was covered with fine gauze. The triatomines were housed at ambient room temperature and high relative humidity, provided by wet cotton on the bottom of the jars in which the vials were stored.

Rhodnius prolixus were infected with trypanosomes by allowing them to feed on mice harboring *T. cruzi*; trypanosomes were found in the feces 21 days later. *Rhodnius neglectus* were inoculated with *T. rangeli* via the hemocoel, and infection was established in the hemolymph 3 days later. Immediately after showing trypanosomes the bugs were fed on viremic animals.

For virus infections, adults and 4th and 5th instar nymphs of *R. prolixus*, *P. herreri*, and *R. neglectus* were fed until engorged on VEE- or

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TABLE 1

Survival of Venezuelan equine encephalitis virus in triatomines

Days after virus ingestion	*R. prolixus*				
	Not punctured	Punctured	*T. cruzi*-infected	Punctured *P. herreri*	*T. rangeli*-infected *R. neglectus*
15	8×10^{1}*	2×10^{2}	–	–	–
17	0†	–	–	–	4×10^{6}
20	–	4×10^{6}	–	–	–
21	0	–	–	–	–
22	–	–	1×10^{6}	4×10^{3}	–
30	0	–	–	–	1×10^{4}
42	–	–	–	8×10^{1}	–
50	0	1×10^{4}	2×10^{1}	–	–
90	0	1×10^{4}	–	–	–
124	–	3×10^{2}	–	–	–

* Virus titer expressed as plaque forming units/bug; 1 or 2 bugs used for each determination.
† Negative for virus.

SLE-viremic mice. Puncturing of the abdomen with a fine needle was performed 1 to 2 hours after engorgement. After viremic blood meals non-punctured *R. prolixus* were kept individually until needed.

At various intervals individual triatomines were macerated in phosphate buffered saline with 0.7% bovine plasma albumin plus 200 units penicillin and 200 μg streptomycin per ml, and centrifuged for 15 minutes at 2,500 × g. Viral content of the supernatant was determined in Vero cells trays,[7] with titers calculated as virus pfu/per bug. Some of these suspensions were also inoculated ic into 1- to 2-day-old mice.

Virus transmission was attempted by allowing individual triatomines to engorge on 1- to 2-day-old mice 15 and 30 days after feeding on viremic hosts. If a mouse became sick or died during the first week, a crude brain antigen was prepared and tested by complement fixation[8] with a reference serum to confirm virus identity.

RESULTS

In non-punctured *Rhodnius prolixus* the virus detected at 15 days was equivalent to 1 log pfu. VEE virus was not found at 17 days or in subsequent examinations (Table 1).

Punctured *R. prolixus* carried VEE virus for the maximum period tested, up to 124 days after ingestion. A drop in titer of 2 to 3 log was evidenced during this interval. *R. prolixus* infected with *T. cruzi* showed a high titer for VEE at 22 days, but by day 50 the titer had diminished to 1 log pfu in the single remaining bug (Table 1). Punctured, clean *P. herreri* also showed a high titer for 22 days which dropped to 1 log pfu of virus in one specimen at 42 days (Table 1).

Almost no loss of VEE titer was found in *T. rangeli*-infected *R. neglectus* 17 days after virus infection, in contrast to titers found in punctured *R. prolixus* 15 days after feeding on viremic hosts. At 30 days one bug had a viremia of 4 log pfu (Table 1).

At 15 days after infection 4 of 8 punctured *R. prolixus* transmitted VEE to mice while feeding; further attempts at 30 days failed, as did six trials at 15 days with non-punctured specimens.

The SLE infections in *R. prolixus* are summarized in Table 2. Punctured bugs yielded approximately 4 log pfu of virus for 15 days; by day 30 virus was detected only by inoculation into mice. No SLE virus was found at 15 or 30 days in non-punctured bugs.

DISCUSSION

Our experiments demonstrate the capacity of punctured triatomines to carry VEE infections

TABLE 2

Survival of St. Louis encephalitis virus in Rhodnius prolixus

Days after virus ingestion	Not punctured	Punctured
15	0	3×10^{4}†
30	0	†

* Virus titer expressed as plaque forming units/bug; 1 or 2 bugs used for each determination.
† Virus recovered by inoculation of mice.

for periods exceeding 4 months. Triatomines are long-lived and require frequent blood meals taken from a spectrum of mammalian and avian hosts. Should virus similarly persist in them in nature, this might resolve questions in the epidemiology of jungle yellow fever, SLE, epizootic VEE, and other arbovirus infections.

The survival of VEE virus in triatomines infected with *T. cruzi* or *T. rangeli* suggests that the virus may naturally cross the barrier of the gut epithelium, pass to the hemocoel, and then be distributed to other tissues. The development of lesions produced by *T. rangeli* in the gut epithelium of triatomines has been shown.[9] Viruses may use such lesions to reach the hemolymph and salivary glands.

Available information (Pedro Galindo, Gorgas Memorial Laboratory, Panama, personal communication) suggests that the observation in laboratory colonies of triatomines sucking blood or lymph from other bugs may indicate a mechanism for transmitting trypanosome infections among wild insect populations. In the case of arboviruses, such transmission could serve both for passing the infection from one bug to another and for producing the puncturing effect necessary for virus replication.

Transmission of VEE by the bite of *R. prolixus* demonstrated the presence of virus in the salivary glands. The reasons for the lack of persistence of the virus, and for its rapid decay after reaching high titers at 3 weeks in *T. cruzi*-infected bugs, are not known. The short survival of SLE in punctured *R. prolixus* may indicate differences in the competence of different species of bugs to carry virus infections.

In further studies, natural associations should be considered. For our experiments we used *R. prolixus* and *R. neglectus*, which are not indigenous to Panama, while the strains of *T. cruzi* and *T. rangeli* were of local origin. The maintenance of triatomines and trypanosome strains in the laboratory probably induces changes in both the bugs and the parasites. These effects may further complicate vector-competence comparisons with naturally-infected bugs.

Finally, it has been shown that *T. cruzi* infection may be transmitted to rodents, and perhaps to other insectivorous vertebrates, by ingestion of infected triatomine bugs (O. Sousa, unpublished data). In view of our current findings, transmission of arboviruses from these insects to vertebrates by the oral route should be investigated. Such a mechanism would represent a new concept in the epidemiology of arboviral infections.

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