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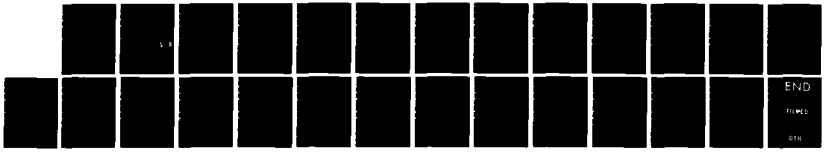

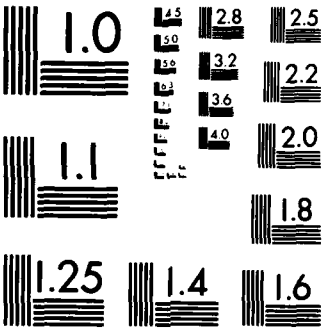

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Studies of the Biology of Phleboviruses in Sandflies

Annual Report

Robert B. Tesh, M.D.

February 1, 1983

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Laboratory reared phlebotomine sand flies were experimentally infected with 11 different phleboviruses to determine their susceptibility following oral and parenteral administration. Most of the viruses replicated in sand flies after intrathoracic inoculation; however, the insects were quite refractory to oral infection. Five of 9 phleboviruses tested were transovarially transmitted in one or more sand fly species. The percentage of infected F_1 offspring produced by parenterally infected female parents ranged from 1.5 - 60%, depending upon the virus type used. These data support the hypothesis that

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some of the phleboviruses are maintained in sand flies by transovarial transmission.

A continuous culture (LL-5) of sand fly (Lutzomyia longipalpis) cells was also established and tested for its ability to support the growth of a number of different arboviruses. Most of the rhabdoviruses, orbiviruses and flaviviruses tested in the LL-5 cells replicated, while most of the alphaviruses and phleboviruses did not. With the exception of Changuinola virus, replication of virus in the sand fly cells occurred without producing cytopathic effect.

Studies of the Biology of Phleboviruses in Sand flies

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Summary Page

Laboratory reared phlebotomine sand flies were experimentally infected with 11 different phleboviruses to determine their susceptibility following oral and parenteral administration. Most of the viruses replicated in sand flies after intrathoracic inoculation; however, the insects were quite refractory to oral infection. Five of 9 phleboviruses tested were transovarially transmitted in one or more sand fly species. The percentage of infected F_1 offspring produced by parenterally infected female parents ranged from 1.5 - 60% depending upon the virus type used. These data support the hypothesis that some of the phleboviruses are maintained in sand flies by transovarial transmission.

A continuous culture (LL-5) of sand fly (Lutzomyia longipalpis) cells was also established and tested for its ability to support the growth of a number of different arboviruses. Most of the rhabdoviruses, orbiviruses and flaviviruses tested in the LL-5 cells replicated, while most of the alphaviruses and phleboviruses did not. With the exception of Changuinola virus, replication of virus in the sand fly cells occurred without producing cytopathic effect.

A. Brief History of the Project

This research project began on 1 September 1980. For the first two years, it was funded as contract DAMD17-80-C-0178, entitled "Studies on the Transovarial Transmission of Phlebotomus Fever Viruses in Sandflies." The original contract terminated on 30 September 1982 and a new contract (DAMD 17-83-C-3002), entitled "Studies on the Biology of Phleboviruses in Sandflies," began on 1 October 1982. This annual report covers the first year of work done under the new contract (DAMD17-83-C-3002); however, it should be noted that this is actually the third year of work on the project, since the overall objectives and personnel have not changed.

During the first 16 months of the project (Sept. 1980 - Dec. 1981), our efforts were focused primarily on establishing laboratory colonies of phlebotomine sandflies. This work took much longer than we had anticipated; and, in retrospect, our original timetable was unrealistic. We found, as many other investigators before us have found, that sandflies are difficult to rear in captivity. These insects are much less prolific than mosquitoes and each generation takes approximately 6 to 8 weeks to develop. Furthermore, the daily care of sandflies is extremely labor intensive. Therefore, new techniques for rearing the insects had to be developed. For these reasons, it was not until December of 1981 that we finally had sandfly colonies of sufficient size and productivity to actually begin experimental studies. We are now able to mass rear sandflies and produce several thousand insects of each species per generation. In fact, our production now exceeds our experimental needs.

B. Sandfly Colonization

At the present time, we maintain six different sandfly colonies in the laboratory. These are Phlebotomus papatasi (geographic strains from India, Israel and Egypt), Lutzomyia longipalpis, Lutzomyia trapidoi and Lutzomyia anthophora. The Indian strain of P. papatasi as well as the L. longipalpis and L. anthophora colonies were started in 1981. These are now well established and are mass produced. The other three colonies which were established during the past year are L. trapidoi, originally obtained from Dr. Byron N. Chaniotis, U. S. Army Medical Department, Panama; the Israel strain of P. papatasi, obtained from Prof. Yosef Schlein, Department of Parasitology, Hadassah Medical School, Jerusalem; and the Egypt strain of P. papatasi received from Dr. John H. Zimmerman, U. S. Naval Medical Research Unit, Cairo. These colonies are now in their second to fourth laboratory generations and production should soon be sufficient for us to begin experimental work with them.

During the past year, we also received a few larval specimens of Phlebotomus chinensis from the Tropical Medical Research Institute, Bejing, China. Unfortunately these specimens arrived in poor condition and did not survive. However, we plan to try to obtain more. Arrangements have also been made to obtain samples of Phlebotomus martini from Dr. Ray Beach, U.S. Army Medical Research Unit, Kenya. This sub-Saharan species is of special interest, since it occurs over a wide area of East Africa where Rift Valley fever is endemic. Negotations are also in progress to obtain Phlebotomus argentipes from India and P. perfiliewi and P. perniciosus from Italy.

C. Experimental Infection of Sandflies

The susceptibility of sandflies to infection with 11 phleboviruses and one vesiculovirus was tested by two methods: direct intrathoracic inoculation and feeding. As shown below, most of the viruses replicated in the insects after inoculation; however, in general the sandflies were quite refactory to oral infection.

1. Rio Grande virus. Table 1 shows the growth of Rio Grande virus in L. anthophora after intrathoracic inoculation. Five sandflies were sampled each day. All of the insects that were tested were infected. Mean virus titers in the flies increased more than 10,000 times by the seventh day after inoculation, indicating that virus replication occurred in the insects.

Some of the flies in this experiment were fed on a hamster on the third day after inoculation and their F_1 progeny were collected and reared to adults. Of 62 F_1 adults tested, 54.8% were infected with Rio Grande virus, indicating that transovarial transmission had occurred (Table 2).

2. Pacui virus. Tables 3 and 4 show the growth of Pacui virus in L. longipalpis and P. papatasi, respectively, following parenteral infection. The virus grew well in both sandfly species, although slightly higher titers were obtained in L. longipalpis. Pacui virus was also transovarially transmitted by both sandfly species; however, the efficiency of vertical transmission among the two species was quite different. Thirty-two percent of the L. longipalplis F_1 adults were infected with Pacui virus, whereas only 2.0% of the P. papatasi F_1 progeny were positive (Table 2). Six transovarially infected F_1 adult L. longipalpis were titrated to determine the amount of Pacui virus present. Titers in these six insects ranged from $10^{4.2}$ to $10^{4.8}$ plaque forming units (PFU) per fly. These titers are comparable to those observed in their female parents (Table 3).

An attempt was also made to orally infect L. longipalpis with Pacui virus by feeding the insects on an artificial blood-virus suspension through a chick skin membrane (Table 5). Although the engorged females contained 3 to 4 logs of virus immediately after feeding, within 24 hours no virus was detectable. Interestingly, a few flies were found to contain virus on days 6 and 7 post-feeding. Since the insects were not sampled beyond the seventh day, it was uncertain whether these results indicated an extremely long eclipse phase in virus replication after oral infection or whether only some of the sandflies were susceptible to oral infection. However, results obtained with Naples, Gabek Forest, Punta Toro and Rift Valley fever viruses (Tables 8,11,16 and 19) suggest the latter case to be true.

3. Naples virus. Tables 6 and 7 show the growth of Naples sandfly fever virus in P. papatasi and L. longipalpis, respectively, following inoculation. Naples virus grew well in P. papatasi but poorly in L. longipalpis. It is noteworthy that Naples virus has been isolated repeatedly from P. papatasi in nature, whereas L. longipalpis is a New World sandfly species and is an unnatural host.

Table 8 shows results of feeding Naples virus to P. papatasi. Although the insects ingested 10^2 to 10^3 PFU of virus, by the fourth day

post-feeding, the virus had disappeared. These data suggest that P. papatasi is relatively refractory to oral infection with Naples virus.

4. Punta Toro virus. The growth of Punta Toro virus in L. longipalpis and P. papatasi is shown in Tables 9 and 10, respectively. Punta Toro virus grew well in L. longipalpis but poorly in P. papatasi. Punta Toro virus and L. longipalpis both occur in the Neotropics, whereas P. papatasi is an Old World sandfly species. These results as well as those with Naples virus suggest that there are differences among sandflies in their susceptibility to various phleboviruses even after inoculation.

Table 11 shows the survival of Punta Toro virus in L. longipalplis after ingestion. Sandflies in this experiment were fed on a viremic hamster. As with Naples virus, most of the flies appeared to be refractory to oral infection.

Attempts to demonstrate transovarial transmission of Punta Toro virus in parenterally infected L. longipalpis were unsuccessful (Table 2); however, it should be noted that L. longipalpis is not the normal vector of this agent. In nature, Punta Toro virus has been associated with L. trapidoi, L. ylephilator and L. sanguinaria.

5. Karimabad virus. Table 12 shows the replication of Karimabad virus in P. papatasi after inoculation. The results are not much different from those obtained with most of the other phleboviruses tested. Since Karimabad virus has been recovered in nature from male P. papatasi, F_1 progeny of experimentally infected females were tested to determine if the virus was transovarially transmitted. As shown in Table 2, 60% of 220 F_1 progeny were infected with the virus. This is the highest transovarial transmission rate we have obtained to date.

6. Sicilian virus. Table 13 shows the growth of Sicilian sandfly fever virus in P. papatasi. Mean virus titers in infected flies increased more than 4 logs during the first three days. It is noteworthy that Sicilian virus also has been recovered from naturally infected P. papatasi of both sexes. Rather surprisingly, however, the transovarial transmission rate of Sicilian virus in experimentally infected P. papatasi (Indian strain) was rather low. Only 2 of 135 (1.5%) F_1 progeny were infected.

7. Saint Floris virus. Table 14 shows the growth of Saint Floris virus in P. papatasi after parenteral infection. This virus was also transovarially transmitted. Of 112 adult F_1 P. papatasi tested, 6.3% contained Saint Floris virus.

8. Rift Valley fever virus. Table 15 shows the growth of Rift Valley fever virus in P. papatasi after inoculation. This work was done at the U.S. Army Medical Research Institute of Infectious Diseases/Fort Detrick in collaboration with Major Alfred Hock. As illustrated in this table, the virus replicated well in P. papatasi following parenteral infection.

In contrast, this same sandfly species was quite refractory to oral infection. Table 16 shows the survival of Rift Valley fever virus in P. papatasi after ingestion. Female sandflies in this experiment were fed on a viremic hamster, circulating $\geq 10^{8.1}$ PFU of virus per ml of blood. The virus titer in the flies immediately after feeding was $10^{4.5}$ PFU/insect;

however, by the third post-feeding day, the virus was no longer detectable in most of the insects. The single infected specimens observed on days 6 and 7 are difficult to explain, but these results are similar to oral infection rates obtained with Pacui and Punta Toro viruses in L. longipalplis (Tables 5 and 11, respectively).

A total of 235 F_1 progeny from parenterally infected female P. papatasi were also cultured for virus. All were negative, suggesting that transovarial transmission of Rift Valley fever virus does not occur in this sandfly species (Table 2). It is noteworthy that P. papatasi parenterally infected with Rift Valley fever virus, were able to transmit the agent by bite to adult hamsters.

9. Gabek Forest virus. Tables 17 and 18 show the growth of Gabek Forest virus in P. papatasi and L. longipalpis after parenteral infection. Although virus replication occurred in both species, higher titers were obtained in P. papatasi. Attempts to demonstrate transovarial transmission of Gabek Forest virus in parenterally infected P. papatasi were unsuccessful (Table 2).

Table 19 shows the survival of Gabek Forest virus in P. papatasi after ingestion. The source of virus in this experiment was a viremic hamster (blood titer = $10^{9.0}$ PFU/ml). Despite the presence of $\geq 10^{4.0}$ PFU per sandfly post-feeding, most of the insects lost all trace of virus. A few females still had virus on days 3,4,5 and 7; but in all cases, virus titers in the infected insects on these days were lower than the titers found immediately after ingestion of the infected blood meal.

10. Itaituba virus. Table 20 shows the growth of Itaituba virus in L. longipalpis.

11. Salehabad virus. Table 21 shows the survival of Salehabad virus in P. papatasi. Unfortunately, the titer of the virus inoculum used in this experiment was relatively high and the mean virus titers in sandflies on subsequent days did not increase. Thus, we cannot say with certainty that Salehabad virus replicates in P. papatasi. Attempts to demonstrate transovarial transmission with this virus-vector combination were also unsuccessful (Table 2).

12. Chandipura virus. For comparison, P. papatasi females were also inoculated with Chandipura virus, a sandfly-associated rhabdovirus of vesicular stomatitis serogroup (genus Vesiculovirus). Replication of Chandipura virus in P. papatasi occurred more rapidly than the phleboviruses (about 4 logs within 24 hours) and then appeared to plateau (Table 22). Transovarial transmission of Chandipura virus in P. papatasi was also demonstrated. Eight percent of the F_1 progeny of parenterally infected female parents contained virus.

In summary, results of our experimental studies to date suggest the following:

(a) That sandflies are susceptible to infection with a variety of phleboviruses when given by intrathoracic inoculation.

(b) That the same sandfly species appear to be fairly refractory to oral infection.

(c) That some phleboviruses are transovarially transmitted in experimentally infected sandflies at relatively high rates.

These observations are all compatible with the hypothesis that some phleboviruses are maintained in nature by transovarial transmission. The poor survival of these viruses after ingestion by sandflies further suggests that viremic vertebrates probably play a minor role in the natural maintenance of these agents and that they are maintained principally by insect to insect transmission.

D. Sandfly Cell Cultures

During the past year, two continuous sandfly cell cultures were developed. These cell lines, designated as LL-5 and PP-9, were started from eggs of Lutzomyia longipalpis and Phlebotomus papatasi, respectively. These are the first sandfly cell cultures to be developed.

The LL-5 culture consists of at least two distinct cell types, which are epithelioid and fibroblastoid in character. The cells are grown in MM/VP_{12} medium and form monolayers on glass or plastic containers. The origin and identity of the LL-5 cells as well as the PP-9 cells have been confirmed by isozyme analysis.

The susceptibility of the LL-5 cells to 29 arboviruses was tested. The viruses examined included representatives of the genera Vesiculovirus, Oribivirus, Flavivirus, Alphavirus, Bunyavirus and Phlebovirus. Approximately 100 plaque forming units (PFU) of each virus were inoculated into 25 cm^2 flasks of the LL-5 cells. After 5 days of incubation at 28°C, the flasks were frozen and the cell harvest titrated in microplate cultures of Vero cells. Virus titers are expressed as the log_{10} of PFU per flask and are given in Table 23. Only 13 of the 29 viruses multiplied in the LL-5 cells. Surprisingly, most of the phleboviruses did not. Of the 14 phleboviruses examined, only Gabek Forest, Anhanga and Icoaraci grew in the cells. Although not shown in Table 23, Rift Valley fever virus was tested at Fort Detrick and was found to replicate in the LL-5 cells. From these results, it was concluded that the LL-5 cells are not particularly useful for in vitro studies of phleboviruses.

The second cell line (PP-9) shows more promise. It is primarily epithelioid in character. Several phleboviruses that have been examined replicate to low titer in the PP-9 cell line. When infected cultures were examined by immunofluorescence, it was found that only about 10-15% of the cells contained specific viral antigen. This observation suggests that only a small proportion of the cells are susceptible to infection, a phenomenon which has been observed before in a number of mosquito cell cultures. Work is now in progress to further characterize the PP-9 cell line and to test its susceptibility to infection with other arboviruses.

E. Publications

Tesh, R.B. and Modi, G.B. Development of a continuous cell line from the sand fly Lutzomyia longipalpis (Diptera: Psychodidae), and its susceptibility to infection with arboviruses. J. Med. Ent. 20: 199-202, 1983.

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Travassos da Rosa, A.P.A., Pinheiro, F.P., Tesh, R.B., Travassos, J.F.S. and N. E. Peterson. Characterization of eight new phlebotomus fever group arboviruses (Bunyaviridae: Phlebovirus) from the Amazon Region of Brazil. Am. J. Trop. Med. Hyg. 32:1164-1171, 1983.

Table 1

Growth of Rio Grande virus in Lutzomyia anthophora after intrathoracic inoculation

Day post-inoculation	Range of titers in positive flies*	Mean titer of positive flies*
0	0.4 - 1.1	0.6
1	0.7 - 1.7	1.3
2	1.7 - 3.4	2.5
3	1.7 - 3.7	2.6
4	2.9 - 3.1	3.1
5	3.4 - 5.0	4.1
6	NT	NT
7	4.3 - 5.7	5.0
8	4.0 - 4.3	4.2

*Virus titers expressed as $\log_{10}$ of tissue culture infectious $dose_{50}$ per insect. Five sandflies were sampled each day.

Table 2

Transovarial transmission rates of selected phleboviruses in sandflies

Virus used to infect parents*	Sandfly species	Number of F_1 progeny tested	Percentage of F_1 progeny infected
Rio Grande	Lutzomyia anthophora	62	54.8
Pacui	Lutzomyia longipalpis	122	32.0
Pacui	Phlebotomus papatasi	51	2.0
Sicilian	Phlebotomus papatasi	135	1.5
Gabek Forest	Phlebotomus papatasi	50	0
Punta Toro	Lutzomyia longipalpis	100	0
Karimabad	Phlebotomus papatasi	220	60.0
Rift Valley fever	Phlebotomus papatasi	235	0
Saint Floris	Phlebotomus papatasi	112	6.3
Salehabad	Phlebotomus papatasi	94	0

*Female parents in these experiments were infected by inoculation.

Table 3

Growth of Pacui virus in Lutzomyia longipalpis after intrathoracic inoculation

Day post-inoculation	Range of titers in positive flies*	Mean titer of positive flies*
0	2.0 - 2.8	2.4
1	4.0 - 4.4	4.1
2	4.6 - 5.4	5.0
3	5.2 - 5.6	5.4
4	4.8 - 5.7	5.3
5	5.0 - 5.4	5.2
6	5.0 - 5.5	5.2
7	4.0 - 5.6	4.9
8	4.9 - 5.4	5.1

*Virus titers expressed as $\log_{10}$ of plaque forming units per insect. Five sandflies were sampled each day.

Table 4

Growth of Pacui virus in Phlebotomus papatasi after intrathoracic inoculation

Day post-inoculation	Range of titers in positive flies*	Mean titers in positive flies
0	<0.7 - 1.7	-
1	1.7 - 3.5	2.7
2	3.0 - 4.6	4.0
3	4.5 - 4.7	4.6
4	4.5 - 5.5	4.8
5	4.3 - 4.8	4.6
6	4.0 - 5.0	4.5
7	3.8 - 5.0	4.4

*Virus titers expressed as $\log_{10}$ of plaque forming units per insect. Five sandflies were sampled each day.

Table 5

Growth of Pacui virus in Lutzomyia longipalpis following ingestion of an artificial blood-virus suspension by the insects

Day post-feeding	Virus titers in insects sampled*
0	3.2, 3.4, 3.5, 3.6, 3.9
1	<0.7, <0.7, <0.7, <0.7, <0.7
2	<0.7, <0.7, <0.7, <0.7, 0.7
3	<0.7, <0.7, 0.7, 1.0, 1.7
4	- - - - -
5	<0.7, <0.7, <0.7, <0.7, <0.7
6	<0.7, <0.7, <0.7, 3.0, 3.4
7	<0.7, 1.6, 3.4, 3.8

*Virus titers expressed as $\log_{10}$ of plaque forming units per insect.

Table 6

Growth of Naples virus in Phlebotomus papatasi after intrathoracic inoculation

Day post-inoculation	Range of titers in positive flies*	Mean titer of positive flies*
0	2.0 - 2.7	2.2
1	2.0 - 2.6	2.3
2	2.8 - 3.4	3.1
3	3.0 - 4.2	3.7
4	3.0 - 4.3	3.5
5	3.0 - 4.0	3.6
6	4.0 - 4.2	4.1
7	3.8 - 4.5	4.2
8	3.8 - 4.5	4.1

*Virus titers expressed as $\log_{10}$ of plaque forming units per insect. Five sandflies were sampled each day.

Table 7

Development of Naples virus in Lutzomyia longipalpis after intrathoracic inoculation

Day post-inoculation	Virus titers in sand flies sampled*
0	2.4, 2.4, 2.7, 2.7, 2.8
1	<0.7, 1.3, 1.4, 1.4
2	1.3, 1.5, 1.5, 1.7, 1.8
3	<0.7, 0.7, 1.2, 1.3, 1.6
4	<0.7, 2.2, 2.3, 2.6, 2.6
5	2.2, 2.3, 2.3, 2.3, 2.4
6	2.2, 2.6, 2.6, 2.8, 3.2
7	<1.7, <1.7, 2.2, 2.3, 3.2

*Virus titers expressed as $\log_{10}$ of plaque forming units per insect.

Table 8

Growth of Naples virus in Phlebotomus papatasi after ingestion*

Day post-feeding	Titers of insects**
0	2.0, 2.6, 2.6, 2.9, 3.2
1	1.7, 2.0, 2.6, 2.6, 2.8
2	<0.7, 1.0, 2.2, 2.2, 2.3
3	<0.7, <0.7, 0.7, 1.0, 1.2
4	<0.7, <0.7, <0.7, <0.7, <0.7
5	<0.7, <0.7, <0.7, <0.7, <0.7
6	<0.7, <0.7, <0.7, <0.7, <0.7
7	<0.7, <0.7, <0.7, <0.7, <0.7

* Flies were fed on a mixture of infected newborn mouse brain and washed human red blood cells through a chick skin membrane.

**Virus titers expressed as $\log_{10}$ of plaque forming units per insect. Five flies were sampled each day.

Table 9

Growth of Punta Toro virus in Lutzomyia longipalpis after intrathoracic inoculation

Day post-inoculation	Range of titers in positive flies*	Mean titer of positive flies*
0	2.0 - 2.3	2.2
1	1.7 - 3.2	2.3
2	3.0 - 3.8	3.4
3	3.9 - 4.2	4.1
4	3.7 - 4.4	4.0
5	3.5 - 4.6	4.1
6	4.0 - 4.7	4.1
7	4.0 - 4.6	4.4

*Virus titers expressed as $\log_{10}$ of plaque forming units per insect. Five sandflies were sampled each day.

Table 10

Growth of Punta Toro virus in Phlebotomus papatasi after intrathoracic inoculation

Day post-inoculation	Virus titer in inoculated insects*
0	0.7, 0.7, 1.4, 1.5, 1.7
1	<0.7,<0.7,<0.7, 0.7, 0.7
2	<1.7,<1.7, 2.0, 2.0, 2.7
3	<1.7,<1.7, 2.2, 2.3, 2.7
4	2.0, 2.6, 2.7, 2.8, 3.0
5	<1.7,<1.7, 1.7, 1.7, 2.5
6	3.2, 3.6, 3.6, 3.6, 3.7
7	<1.7,<1.7, 2.0, 2.2, 2.7

*Virus titers expressed as $\log_{10}$ of plaque forming units per insect. Five sandflies were sampled each day.

Table 11

Survival of Punta Toro virus in Lutzomyia longipalpis after ingestion. Sandflies were fed on a hamster infected with Punta Toro virus. Titer of hamster's viremia = $10^{7.8}$ PFU/ml

Day post-feeding	Virus titers in insects sampled*
0	2.4, 2.8, 2.8, 3.9, 4.2
1	2.0, 2.8, 3.0, 3.0, 3.3
2	<0.7, <0.7, <0.7, 0.7, 0.7
3	<0.7, <0.7, 0.7, 1.3, 1.7
4	<0.7, <0.7, <0.7, <0.7, <0.7
5	<0.7, <0.7, <0.7, <0.7, 0.7
6	<0.7, 0.7, 1.0, 1.0, 1.4
7	<0.7, <0.7, <0.7, <0.7, 0.7

*Virus titers expressed as $\log_{10}$ of plaque forming units per insect.

Table 12

Growth of Karimabad virus in Phlebotomus papatasi after intrathoracic inoculation

Day post-inoculation	Range of titers in positive flies*	Mean titer of positive flies*
0	2.0 - 2.6	2.2
1	2.5 - 2.8	2.6
2	3.6 - 4.5	4.2
3	3.4 - 4.5	3.9
4	4.0 - 5.2	4.6
5	4.0 - 4.6	4.4
6	4.0 - 4.8	4.3
7	4.0 - 4.5	4.3
8	4.0 - 4.8	4.3

*Virus titers expressed as $\log_{10}$ of plaque forming units per insect. Five sandflies were sampled each day.

Table 13

Growth of Sicilian virus in Phlebotomus papatasi after intrathoracic inoculation

Day post-inoculation	Range of titers in positive flies*	Mean titer of positive flies*
0	<1.0	<1.0
1	<1.7 - 2.0	1.8
2	2.0 - 4.0	3.0
3	3.7 - 4.7	4.4
4	3.2 - 5.4	4.0
5	3.5 - 4.2	3.9
6	3.6 - 4.7	4.3
7	3.5 - 4.8	4.3

*Virus titers expressed as $\log_{10}$ of plaque forming units per insect. Five sandflies were sampled each day.

Table 14

Growth of Saint Floris in Phlebotomus papatasi after intrathoracic inoculation

Day post-inoculation	Range of titers in positive flies*	Mean titers in positive flies*
0	2.0 - 2.6	2.4
1	<1.7	<1.7
2	2.8 - 3.7	3.2
3	3.0 - 4.2	3.5
4	3.0 - 3.8	3.4
5	3.6 - 4.0	3.8
6	4.0 - 4.3	4.1
7	3.6 - 4.3	3.9

*Virus titers expressed as $\log_{10}$ of plaque forming units per insect. Five sandflies were sampled each day.

Table 15

Growth Of Rift Valley Fever Virus In Phlebotomus Papatasi After Intrathoracic Inoculation

Day post inoculation	Number infected/ number sampled	Range of titers in infected flies*	Mean titer in infected flies*
0	10/10	1.7 - 2.7	2.2
1	10/10	2.4 - 4.2	3.2
2	10/10	4.0 - 5.6	4.7
3	10/10	4.7 - 5.6	5.3
4	10/10	4.8 - 5.6	5.4
5	10/10	4.5 - 5.6	5.4
6	10/10	4.6 - 5.7	5.2
7	4/4	5.2 - 5.6	5.4
14	7/7	4.8 - 5.7	5.0

*Virus titers expressed as $\log_{10}$ of plaque forming units per insect.

Table 16

Growth Of Rift Valley Fever Virus In Phlebotomus Papatasi After Blood Feeding On A Viremic Hamster*

Day post blood feeding	Number infected/ number sampled	Range of titers in infected flies**	Mean titer in infected flies**
0	5/5	4.2 - 4.7	4.5
1	5/5	3.9 - 4.3	4.1
2	5/5	3.4 - 3.8	3.6
3	1/5	2.0	2.0
4	1/5	1.0	1.0
5	0/5	0	0
6	1/5	5.2	5.2
7	1/5	2.2	2.2
8	0/5	0	0

*Pre- and post-exposure blood samples in the infected hamster were $10^{8.1}$ and $10^{8.7}$ PFU/ml of blood, respectively.

** Titers expressed as $\log_{10}$ of plaque forming units per insect.

Table 17

Growth of Gabek Forest virus in Phlebotomus papatasi after intrathoracic inoculation

Day post-inoculation	Range of titers in positive flies*	Mean titer of positive flies*
0	0.5 - 2.5	1.8
1	2.3 - 2.6	2.5
2	2.7 - 3.2	3.0
3	4.0 - 4.5	4.1
4	4.0 - 5.5	4.9
5	4.6 - 5.2	4.9
6	4.6 - 5.3	5.1
7	4.8 - 5.3	5.1

*Virus titers expressed as $\log_{10}$ of plaque forming units per insect.

Table 18

Growth of Gabek Forest virus in Lutzomyia longipalpis after intrathoraic inoculation

Day post-inoculation	Range of titers in positive flies*	Mean titer of positive flies*
0	2.6 - 3.0	2.8
1	1.2 - 2.2	1.6
2	1.7 - 2.3	2.0
3	2.7 - 3.6	3.1
4	2.7 - 3.5	3.2
5	2.8 - 3.6	3.3
6	3.0 - 4.2	3.6
7	3.7 - 4.3	4.0

*Virus titers expressed as $\log_{10}$ of plaque forming units per insect. Five sandflies were sampled each day.

Table 19

Survival of Gabek Forest virus in *Phlebotomus papatasi* after ingestion. Sandflies were fed on a hamster infected with Gabek Forest virus. Titer of hamster's viremia = $10^{9.0}$ PFU/ml

Day post-feeding	Virus titers in insects sampled*
0	4.3, 4.5, 4.5, 4.8, 4.8
1	4.3, 4.5, 4.9, 5.0, 5.0
2	<0.7, <0.7, 1.0, 3.0 (only 4 flies tested)
3	<0.7, <0.7, <0.7, 2.4, 3.7
4	<0.7, <0.7, 2.9, 3.7, 4.9
5	<1.7, 2.0, 2.0, 3.2, 3.7
6	<1.7, <1.7, <1.7, <1.7, <1.7
7	<1.7, <1.7, 2.5, 4.0, 4.2

*Virus titers expressed as $\log_{10}$ of plaque forming units per insect.

Table 20

Growth of Itaituba virus in *Lutzomyia longipalpis* after intrathoracic inoculation

Day post-inoculation	Virus titers in insects sampled*
0	<0.7, 1.2, 1.3, 1.4, 1.7
1	<0.7, <0.7, 0.7, 0.7, 0.7
2	<0.7, <0.7, <0.7, <0.7, 0.7
3	<1.7, 1.7, 2.6, 2.6, 3.7
4	2.5, 2.5, 3.0, 3.0, 3.3
5	<0.7, 2.4, 2.6, 2.9, 4.2
6	<0.7, 3.0, 3.6, 3.6, 3.7
7	2.7, 3.0, 3.2, 4.0, 4.5

*Virus titers expressed as $\log_{10}$ of plaque forming units per insect. Five sandflies were sampled each day.

Table 21

Growth of Salehabad virus in Phlebotomus papatasi after intrathoracic inoculation

Day post-inoculation	Range of titers in positive flies*	Mean titer of positive flies*
0	2.8 - 4.0	3.6
1	3.5 - 3.6	3.6
2	3.3 - 3.9	3.6
3	3.2 - 3.6	3.4
4	3.0 - 3.6	3.4
5	2.9 - 3.4	3.1
6	3.0 - 4.0	3.3
7	3.0 - 3.5	3.2

*Virus titers expressed as $\log_{10}$ of plaque forming units per insect. Five sandflies were sampled each day.

Table 22

Growth of Chandipura virus in Phlebotomus papatasi after intrathoracic inoculation

Day post-inoculation	Range of titers in positive flies*	Mean titer of positive flies*
0	1.2 - 2.0	1.5
1	5.0 - 5.8	5.4
2	5.2 - 6.4	5.6
3	4.6 - 5.3	5.0
4	3.8 - 4.8	4.4
5	4.3 - 4.9	4.6
6	3.6 - 5.2	4.5

*Virus titers expressed as $\log_{10}$ of plaque forming units per insect. Five sand flies were sampled each day.

Table 23

Growth of selected arboviruses in the LL-5 cell line

Virus identification	Strain	Taxonomic group (genus)	Titer*	Arthropod association
vesicular stomatitis (Indiana)	VP-98F	Vesiculovirus	7.5	sandfly
Chandipura	I653514	"	6.5	sandfly
Isfahan	91025-C	"	5.4	sandfly
Jurona	BeAr40578	"	5.9	mosquito
Poona 733646	Poona 733646	Rhabdovirus (unclassified)	6.1	sandfly
Kununurra	Or 194	"	5.6	mosquito
Klamath	M 1056	"	1.4	unknown
Changuinola	BT-436	Orbivirus	6.9	sandfly
Colorado tick fever	Florio	"	1.4	tick
Kemerovo	EgAn 1169-61	"	4.3	tick
St. Louis encephalitis	Porton	Flavivirus	3.5	mosquito
West Nile	Egypt 101	"	4.3	mosquito and tick
Chikungunya	Ross	Alphavirus	1.9	mosquito
Ross River	T 48	"	1.7	mosquito
Cache Valley	Holden	Bunyavirus	1.4	mosquito
Naples sandfly fever	Naples	Phlebovirus	1.4	sandfly
Sicilian sandfly fever	Sicilian	"	1.4	sandfly
Punta Toro	D-40210A	"	1.4	sandfly
Chagres	JW 10	"	1.4	unknown
Gabek Forest	SudAn 754-61	"	5.7	unknown
Pacui	BeAn 27326	"	1.4	sandfly
Anhanga	BeAn 46852	"	4.6	unknown
Icoaraci	BeAn 24262	"	3.5	sandfly and mosquito
Arumowat	Ar 1284-64	"	1.4	mosquito
Joa	BeAr 371637	"	1.4	sandfly
Aguacate	VP-175A	"	1.4	sandfly
Salehabad	I-81	"	1.4	sandfly
Candiru	BeH 22511	"	1.4	unknown
Karimabad	I-58	"	1.4	sandfly

*Virus titer expressed as $\log_{10}$ of plaque forming units (PFU) on the fifth day after inoculation with $10^{2.0}$ PFU.

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