A COMPARISON OF SUCKLING MOUSE AND MOSQUITO SUSCEPTIBILITY TO INFECTION BY THE BUNYAMWERA GROUP ARBOVIRUSES[1]

CURTIS G. HAYES[2] AND EDWIN C. CORRISTAN
Biological Sciences Laboratories, Fort Detrick, Frederick, Maryland 21701

INTRODUCTION. The suckling mouse has been generally accepted as one of the most sensitive assay systems, by intracerebral (IC) inoculation, for the study of mosquito-borne viruses in the laboratory (Chamberlain, 1968). It is often used for the initial isolation of virus from field-collected mosquitoes and for determining the ability of laboratory infected mosquitoes to support virus replication and to transmit virus by bite.

In studying the laboratory infection of mosquitoes by ingested virus, several investigators have shown that the midgut of the mosquito exhibits a "threshold barrier" to the initiation of generalized infection (Chamberlain, 1968). To overcome this "gut barrier" phenomenon, parenteral inoculation techniques have been used (Hurlbut and Thomas, 1960, 1969; Mussgay and Suarez, 1962; Lam and Marshall, 1968; Rozeboom and Kassira 1969; Schaffer and Scherer, 1971). These techniques permit the inoculum to be introduced directly into the hemocoele, bypassing the midgut, and as a result reduce the concentration of virus required for infection (Chamberlain and Sudia, 1961).

In the present study, inoculation techniques were used to compare the relative susceptibility of mosquitoes and suckling mice to infection with three arboviruses. We felt that such comparative data on these two *in vivo* host systems would be useful in the study of vector-virus relationships.

MATERIALS AND METHODS. Three arboviruses of the Bunyamwera group, Guaroa, Germiston, and Bunyamwera, were used in this study. Guaroa virus was obtained from the National Cancer Institute. Its history was unknown. Germiston and Bunyamwera virus were obtained from stock seeds maintained in our laboratory. Germiston virus, strain SAAR 1050, had received 11 suckling mouse brain passages and Bunyamwera, original strain, had received 40 suckling mouse brain passages. All three viruses were passed once in Vero tissue cells to produce a sufficient quantity of working seed (WS).

Three species of mosquitoes and 1–3 day old Swiss mice were used as hosts. The species of mosquitoes used were *Aedes aegypti* (L), *Aedes triseriatus* (Say), and *Psorophora ferox* (Humboldt). The *A. aegypti* were obtained originally from the Rockefeller Foundation and had been maintained in our laboratory colony for a number of years. The *A. triseriatus* colony had been maintained in culture since 1952 and the *P. ferox* colony was established in 1969 from eggs furnished by H. C. Chapman, U. S. Department of Agriculture, ARS, Lake Charles, Louisiana.

Each virus WS was titrated in suckling mice (SM) to determine the intracerebral median lethal doses per ml ($ICLD_{50}$/ml), and assayed for infectivity in mosquitoes to determine the intrathoracic median infective doses per ml ($ITID_{50}$/ml). The $SMICLD_{50}$/ml and the Mosq. $ITID_{50}$/ml were used as directed indicators of the comparative host susceptibility to each virus.

For the mice, 10-fold serial dilutions of

---

[1] In conducting the research reported herein, the investigators adhered to *Guide for Laboratory Animal Facilities and Care* established by the Committee on *Guide for Laboratory Animal Facilities and Care* of the Institute of Laboratory Animal Resources, NAS-NRC.

[2] Present address: School of Medicine, Department of Epidemiology and Public Health, Yale Univ., New Haven, Connecticut, 06510.

each virus WS were inoculated IC in the volume of .02 ml/mouse and end points of infection recorded. An IC infective dose was assumed to be a lethal dose and death was used as the criterion for infection.

For the mosquitoes, four 10-fold serial dilutions were made of each virus WS and inoculated into separate batches of 5–10 day old virgin females of each species. Individual mosquitoes received approximately .001 ml of inoculum intrathoracicly. This dose was equivalent to 10–300 $SMICLD_{50}$.

Injections were made under a dissecting scope. Capillary tubes, drawn under flame, were used as needles.

After inoculation, the mosquitoes were held for 10 days at 26±2°C and 80±5 percent relative humidity (RH) and fed on a 5 percent sucrose solution. The mosquitoes surviving this period were frozen at −20° C and held for viral assay.

Forty mosquitoes of each species, 10 per WS dilution, were assayed qualitatively for infection for each virus. Four mosquitoes of each species, inoculated with the lowest dilution from each WS, were also titrated to check quantitatively for viral replication.

These mosquitoes exhibited no readily visible external signs of infection; therefore, subinoculation of SM was used as an assay system for detection of viral infectivity and replication. The mosquitoes were triturated individually using a micro-mortar and pestle with 1 ml of diluent (Eagle's Minimum Essential Medium with 10 percent fetal calf serum and antibiotic solution). This suspension was used directly to assay qualitatively for infection. One litter of six mice were inoculated per mosquito, .02 ml IC/mouse. Tenfold serial dilutions of this suspension were made for titration in SM. All end points of infectivity were calculated by the method of Reed-Muench (1938).

RESULTS. The results of the experiments to assay for virus infectivity in the mosquitoes are contained in Table 1. Overall, of the three species tested, *A. aegypti* appears to be slightly more susceptible to infection with these viruses.

Table 2 contains the quantitative data comparing the susceptibility of the mosquitoes and SM to infection with the three arboviruses. With the Guaroa virus WS, no significant difference (F test; $P < 0.05$) existed between the Mosq. $ITID_{50}$ and the $SMICLD_{50}$/ml. For the Bunyamwera and Germiston virus working seeds, the $SMICLD_{50}$/ml was significantly greater (F test $P < 0.05$) than the Mosq. $ITID_{50}$/ml. With these latter two viruses, the $SMICLD_{50}$/ml ranged from 0.7 to 2 logs greater than the Mosq. $ITID_{50}$/ml.

TABLE 1.—Susceptibility of three species of mosquitoes to parenteral infection with three arboviruses of the Bunyamwera group.[a]

Mosquito species	Virus	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
Aedes aegypti	Guaroa	....[b]	9/10[c]	9/10	2/10	0/10	....
	Bunyamwera	....	10/10	4/10	1/10	0/10	....
	Germiston	....	....	8/10	1/10	1/10	0/10
Aedes triseriatus	Guaroa	....	6/10	4/10	1/10	0/10	....
	Bunyamwera	....	6/10	0/10	0/10	0/10	....
	Germiston	....	....	8/10	2/10	0/10	0/10
Psorophora ferox	Guaroa	....	10/10	5/10	2/10	0/10	....
	Bunyamwera	7/10	4/10	0/10	0/10	....	....
	Germiston	....	7/10	2/10	0/10	0/10	....

[a] Volume of mosquito inoculum 0.001 ml; 10-day postinoculation incubation at 26.5 C; mosquitoes assayed for infection in suckling mice.

[b] — = not done.

[c] Number infected/number screened.

TABLE 2.—Results of infection assay and titration experiments on three species of mosquitoes infected with three arboviruses of the Bunyamwera group.

Mosquito species	Virus	Working seed $SMICLD_{50}$/ml	Working seed mosquito $ITID_{50}$/ml[a]	Working seed $SMICLD_{50}$ inoculated per mosq. at lowest dilu.	Range of titers ($SMICLD_{50}$/mosq.) 10-day post-inoculation	Mean titer 10-day post-inoculation[c]
Aedes aegypti	Guaroa	7.9[b]	8.5	0.9	4.3–4.7	4.6 x
	Bunyamwera	8.6	7.9	1.6	6.0–6.2	6.1 y
	Germiston	9.2	8.4	1.2	5.7–6.2	6.0 y
Aedes triseriatus	Guaroa	7.9	7.6	0.9	4.9–5.4	5.2 x
	Bunyamwera	8.6	7.2	1.6	5.4–6.1	5.8 y
	Germiston	9.2	8.5	1.2	5.5–6.7	6.3 y
Psorophora ferox	Guaroa	7.9	8.2	0.9	4.3–5.3	4.9 x
	Bunyamwera	8.6	6.6	2.6	5.2–6.0	5.5 xy
	Germiston	9.2	7.4	2.2	5.1–6.9	6.2 y

[a] Mosquitoes were assayed for infectious virus and titered in suckling mice. It was assumed that for suckling mice an ic infective dose was equivalent to a lethal dose.

[b] $SMICLD_{50}$ and $MITID_{50}$ $\log_{10}$.

[c] Means for each mosquito species with the same letter suffix were not significantly different according to the F test ($P<0.05$).

Also presented in Table 2 are the results of the mosquito titrations in SM, that show all three viruses capable of replicating in each mosquito species during the 10-day postinoculation period. The quantitative increase of 10-day postinoculation titers versus the initial injected dose ranged from 2.6 up to 5.5 logs.

Mosquitoes inoculated with Guaroa virus consistently exhibited the lowest 10-day postinoculation titers, showing a significant difference from the titers of those inoculated with Germiston in all three species. There was a less pronounced difference between the titers of mosquitoes inoculated with Guaroa WS and those inoculated with Bunyamwera WS except for *A. aegypti.* No significant difference existed between mosquitoes inoculated with Germiston WS and those inoculated with Bunyamwera WS.

DISCUSSION. It would appear from these results, that the suckling mouse as an assay system for infectivity of these Bunyamwera group arboviruses, is equal to or more sensitive than, any of the mosquito species tested. Guaroa was the only virus demonstrating equal infectivity for the mosquitoes and suckling mice. A similar experiment was conducted by Hurlbut (1965) comparing the susceptibility of *Culex univittatus* Theobald and adult mice to infection with West Nile virus by inoculation techniques. In contradistinction, his results indicated that 10-fold less virus was required to infect the mosquitoes compared to the adult mice.

Even though Guaroa virus exhibited the greatest infectivity for all three species, its 10-day postinoculation titer in mosquitoes was lowest. This would seem to indicate that no positive correlation exists between the initial susceptibility to infection and the extent of viral replication in the mosquitoes tested.

In relation to the results obtained, an interesting comparison could be made between the susceptibility to infection via parenteral inoculation and ingestion of virus to determine if the same order of susceptibility would be maintained in these mosquitoes. The magnitude of the 10-day postinoculation titers in relation to actual biological transmission of the viruses by the mosquitoes could also be determined.

SUMMARY. The comparative susceptibility of mosquitoes and suckling mice to infection by inoculation with three Bunyamwera group arboviruses was determined. The three viruses used were Guaroa, Germiston, and Bunyamwera. One to 3 days old Swiss mice and three species of mosquitoes, *A. aegypti, A. triseriatus,* and *P. ferox,* were used as hosts.

The results of experiments comparing the $SMICLD_{50}$/ml and the Mosq. $ITID_{50}$/ml of each virus showed that the suckling mouse was as susceptible as the mosquitoes to infection with the three viruses, or more so. Guaroa was the only virus that was equally infectious for both hosts.

Mosquito titration experiments using the SM as an assay system showed that all three viruses multiplied in all three species of mosquitoes during the 10-day postinoculation period. Low 10-day postinoculation titers obtained for Guaroa virus would seem to indicate that no positive correlation can be made between the initial susceptibility to infection and the magnitude of the final postinoculation titers in the mosquitoes.

References

Chamberlain, R. W. and Sudia, W. D. 1961. Mechanism of transmission of viruses by mosquitoes. Ann. Rev. of Entomol. 6:371–390.

Chamberlain, R. W. 1968. Arboviruses, the arthropod-borne animal viruses. Current topics in Microbiol. and Immunol. 42:38–58.

Hurlbut, H. S. 1956. West Nile infection in arthropods. Am. J. Trop. Med. Hyg. 5:76–85.

Hurlbut, H. S. and Thomas, T. I. 1960. The experimental host range of the arthropod-borne animal viruses in arthropods. Virology 12:391–407.

Hurlbut, H. S. and Thomas, T. I. 1969. Further studies on the arthropod host range of arboviruses. J. Med. Ent. 6:423–427.

Lam, K. S. K. and Marshall, I. D. 1968. Dual infections of *Aedes aegypti* with arboviruses.

I. Arboviruses that have no apparent cytopathic effect in the mosquito. Am. J. Trop. Med. Hyg. 17:625–636.
Mussgay, M. and Suarez, O. 1962. Studies with a pathogenic and an attenuated strain of Venezuelan equine encephalitis virus and *Aedes aegypti* (L.) mosquitoes. Arch. Ges. Virusforsch 12:387–392.
Reed, L. J. and Meunch, H. 1938. A simple method of estimating fifty per cent end points. Amer. J. Hyg. 27:493–497.
Rozeboom, L. E. and Kassira, E. N. 1969. Dual infections of mosquitoes with strains of West Nile virus. J. Med. Ent. 6:407–411.
Schaffer, P. A. and Scherer, W. F. 1971. Stability of virulence and plaque size of Venezuelan encephalitis virus with passage in mosquitoes (*Aedes aegypti*). Amer. J. Epidem. 93:68–74.

SEASONAL ABUNDANCE OF ADULT *AEDES AEGYPTI* IN DJAKARTA, INDONESIA [1]

P. F. D. VAN PEENEN,[2] SOEROTO ATMOSOEDJONO,[2]
J. C. LIEN [3] AND SULIANTI SAROSO, J.[4]

ABSTRACT. Adult *Aedes aegypti* mosquitoes were collected systematically from houses in four areas of Djakarta, Indonesia during 1970–71. This species was easily collected from different parts of the city during all months of the year. There was considerable variation in monthly abundance of adult female *Aedes aegypti* from area to area, but there was no clearcut seasonal pattern, despite a distinct rainy season.

INTRODUCTION. Seasonal dengue outbreaks occur in Djakarta, Indonesia, often causing dengue hemorrhagic fever (DHF) in children (Kho *et al*, 1969). *Aedes aegypti* mosquitoes are presumed to be dengue vectors here, as they are elsewhere in Southeast Asia (Halsted, 1966; Russell *et al*, 1969). Studies of the distribution and seasonal abundance of this mosquito in Djakarta were therefore undertaken in 1970–71 with results noted herein.

METHODS. Preliminary surveys, and data from other entomological studies at this Detachment showed, as expected, that *Aedes* adults were active only during daylight, and were rarely collected out-of-doors. In addition, a peak of biting activity consistently occurred between 0900 and 1200.

Collections of *Aedes* were therefore planned in the mornings: a team of three men was trained to collect mosquitoes, and four areas considered representative of geographic and socio-economic sectors of the city were chosen. Each area was visited on the same day once weekly. The three men collected independently for 10 minutes in six different houses using a flashlight and aspirator tube commencing at about 0830. Afterwards, they worked for 5 minutes in each of another six houses in the area using sweep nets.

Data were kindly analyzed by Mr. Richard See, Head of the Department of Data Processing in Taipei, using IBM data cards and computer programs.

DESCRIPTION OF COLLECTING SITES. Djakarta, the capital of Indonesia, is a teeming city of almost five million people,

[1] From Research project MR 005.09.01 0121B6-HJ, Bureau of Medicine and Surgery, Washington, D.C. The opinions and assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the Naval service at large.

[2] Naval Medical Research Unit No. 2 Djakarta Detachment, APO San Francisco 96356.
[3] Naval Medical Research Unit No. 2, APO San Francisco 96263.
[4] Chairman, National Institute for Medical Research, Djalan Pertjetakan Negara I, Djakarta, Indonesia.