

## ST. LOUIS ENCEPHALITIS OUTBREAK IN CAMERON COUNTY, TEXAS

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In late July and August of 1957 we were called upon to offer entomological assistance in the investigation and suppression of an outbreak of encephalitis in Cameron County, Texas, three years following a major epidemic of the same disease in the adjacent county of Hidalgo (Chin, *et al.*, 1957). Epidemiologists from the Texas State Department of Health Communicable Disease Division and Epidemic Intelligence Officers, Communicable Disease Center, U. S. Public Health Service, reported a total of 119 clinically diagnosed cases; a majority of those satisfactorily tested serologically were confirmed as St. Louis encephalitis in the Virus and Rickettsial Section, Texas State Department of Health Laboratory.

A total of 15 entomological working days were spent in Cameron County from July 31 to August 16, 1957, in connection with the epidemic. Emphasis was placed on the demonstration of larval breeding sites and adult mosquito concentrations for the benefit of control crews under the supervision of personnel from the Sanitary Engineering Division, Texas State Department of Health, and the collection of adults to be utilized in virus isolation attempts. Particular attention was directed to the Harlingen-San Benito area, in the northern half of the County, where the cases were concentrated. Approximately 75,000 people reside in the affected section.

The elevation of Cameron County ranges from sea level to 60 feet and marked seasonal changes do not occur. The climate is semitropical, with an average January temperature of 60° F., 84° F. in July and an annual mean of 77° F. The average rainfall is 29.55 inches with most of it falling during the spring or fall. The economy is closely associated with highly industrialized, irrigation agriculture. Principal winter products are citrus and

vegetables with cotton of primary importance during the summer.

Extremely dry conditions prevailed at the time of the epidemic as little rain fell in July and none in August. Harlingen reported 4.34 inches in April, 2.32 in May, 6.67 in June and .07 in July. During the investigation the cotton crop had matured and was being picked, resulting in irrigation activities being at a minimum. Irrigation was noted in a few localized areas such as nurseries and the fairways of golf courses, providing breeding media for limited numbers of *Psorophora confinnis* and *Aedes sollicitans*.

*Culex quinquefasciatus* was the prevalent species, being produced in quantity in urban areas in connection with the municipal procurement of water and the disposal of sewage and industrial wastes. Breeding foci included such sites as the settling beds of an unused sewage plant, the effluent from an operating sewage plant, standing water adjacent to a city well and pooled water in storm sewers. In suburban and rural areas, *Culex quinquefasciatus* could be found around most homes inspected, developing in such places as septic tanks or septic tank effluents, and stock watering containers.

The irrigation canals which crisscross the Valley were found to be well maintained and not breeding mosquitoes. However, each of the canals is provided with an adjacent drainage ditch. For the most part, these ditches were clogged with cattails and other emergent vegetation and when provided with seepage irrigation water were breeding *Anopheles quadrimaculatus*, *Culex erraticus* and *C. coronator*.

Adult mosquitoes for virus studies were collected alive by hand from such daytime retreats as chicken houses and calf sheds, by light traps equipped with bobbinet

bags instead of filling jars, while feeding, and by sweeping vegetation with insect nets.

The total number of adult mosquitoes collected was 1,900. Of this number 1,619 (85.2 percent) were *Culex quinquefasciatus*. The remainder (14.8 percent) was composed of 146 *Anopheles quadrimaculatus*, 60 *Aedes sollicitans*, 52 *Psorophora confinis*, 12 *Culex erraticus*, 5 *Aedes vexans*, 3 *Culex tarsalis* and 3 *Culex coronator*.

April, 1958, 216 *Culex quinquefasciatus*, 40 *Anopheles quadrimaculatus*, 34 *Aedes vexans*, 3 *Aedes sollicitans*, 1 *Culex tarsalis* and 1 *Culex coronator*; and May, 1958, 563 *Culex quinquefasciatus*, 366 *Anopheles quadrimaculatus*, 76 *Aedes vexans* and 17 *Culex erraticus*.

LABORATORY FINDINGS. Mosquitoes collected for virus isolation attempts were pooled according to place of collection, date of collection and species. In preparing the pools of mosquitoes for animal

TABLE 1.—Mosquitoes collected for virus testing in Cameron County, Texas, August 1957

Species	Number of mosquitoes	Number of pools tested	Number of positive pools (SLE)
<i>Culex quinquefasciatus</i>	1,619	27	8
<i>Anopheles quadrimaculatus</i>	146	4	—
<i>Aedes sollicitans</i>	60	2	—
<i>Psorophora confinis</i>	52	2	—
<i>Culex erraticus</i>	12	1	—
<i>Aedes vexans</i>	5	0	—
<i>Culex tarsalis</i>	3	0	—
<i>Culex coronator</i>	3	0	—
Totals	1,900	36	8

The *Culex quinquefasciatus* were collected chiefly from chicken houses, sewage plants and storm sewers, while the *Anopheles quadrimaculatus* were almost all found in cattle sheds. The *Aedes* and *Psorophora* were taken in a few areas being irrigated. As many of the mosquitoes contained freshly ingested blood when captured, they were held alive for 24 to 48 hours to allow for digestion of the blood to insure that viral isolates reflected actual infections rather than merely the viremic state of a host animal or animals. The mosquitoes were anesthetized, sealed under glass, frozen and subsequently taken on dry ice to the Austin Laboratory of the Texas State Department of Health for identification and virus isolation attempts.

Subsequent to the epidemic the following mosquitoes were collected in Cameron County for virus isolation attempts: December, 1957, 1,083 *Culex quinquefasciatus*; January, 1958, 682 *Culex quinquefasciatus*;

inoculation pre-chilled sterile mortars and pestles were used for grinding the mosquitoes which were then suspended in 20 percent normal egg yolk (from six or seven day-old-chick embryos) in phosphate buffer (pH 7.4-7.6) containing 1,000 units of penicillin and 250 micrograms of streptomycin per milliliter. One milliliter of suspending fluid was used for each 20 to 25 mosquitoes in the pool. After thorough mixing of the suspension, aliquots were sealed under glass and stored in a deep freeze for any future studies indicated. The remaining portion was allowed to stand in the cold for approximately one hour before centrifugation for 15 minutes at 2500 r.p.m. The resulting supernatant was then used for inoculation of white mice by the intracerebral route. Five mice were used for each mosquito pool.

Inoculated mice were observed daily for signs of encephalitis virus infections. Sick mice were usually sacrificed. Brains

of mice sacrificed and of those found dead were cultured in thioglycolate medium to check for bacterial contamination. Brains of such animals were also passaged to a second group of mice to insure that a virus was present. The sources of viruses so isolated were proved by re-isolation from the original mosquito suspensions.

If all mice inoculated with a given specimen showed no signs of illness by the ninth day, they were sacrificed and their brains used to make a blind passage to another group of mice which was examined for signs of illness for two weeks before the specimen was considered negative for virus. However, no viruses were isolated in any of the second mouse passages.

Eight different pools of *Culex quinquefasciatus* collected the middle of August 1957, yielded virus. One pool was col-

lected from the pens of a pet farm near Harlingen which specialized in birds and chickens. A storm sewer tunnel (plate 1) in Harlingen was the site where six positive pools were collected. The eighth positive pool was collected from a chicken house adjacent to a residence near San Benito. The first attempts to identify these isolated was by the use of serum-virus neutralization tests in mice using antiserum to St. Louis encephalitis (SLE) virus since complement-fixation tests on serums from human cases of encephalitis had shown SLE to be active in the area. Although this serum had the capacity to neutralize 1,000 mouse LD/50's of our laboratory strain of SLE virus, it did not prove to be adequate to identify these viruses. Further studies within one of these viruses showed ten-day-old chick embryos to be susceptible when inoculated via the amni-



PLATE 1.—Storm sewer tunnel in the outskirts of Harlingen, Texas, from which St. Louis encephalitis isolates were obtained from six pools of *Culex quinquefasciatus*

otic cavity. This virus also produced a viremia in one-day-old chicks but it did not kill them.

Continued studies of this one particular isolate were accomplished by the production of antiserum in guinea pigs and mice using live virus vaccine in mouse brain suspensions. Embryo suspensions were used to vaccinate young adult chickens. Antiserum produced in these three types of animals when tested in serum-virus neutralization tests in mice was shown to neutralize the homologous virus and our stock SLE virus but not western equine encephalitis virus. Furthermore the other seven isolates were also neutralized by the mouse antiserum. Finally then it was proved that all eight virus isolates from these mosquitoes were SLE virus.

No viral isolates were obtained from the 3,082 mosquitoes collected from Cameron County in December, 1957, January, April and May, 1958 and processed in 61 pools.

**DISCUSSION.** *Culex quinquefasciatus* was shown to be the prevalent mosquito during the investigation of the 1957 outbreak of St. Louis encephalitis in Cameron County, as was the case in the adjacent county of Hidalgo during the 1954 epidemic of this disease. Local residents were almost unanimously of the opinion that mosquito populations were low, indicating that sizeable numbers of *C. quinquefasciatus* can be present without being evident to private citizens or even local health officials. Too often, control measures are closely connected with the investigation of complaints—and complaints in the study area usually result from flood water *Aedes* and *Psorophora* attacks. *C. quinquefasciatus*, with its preference for water rich in organic matter, whether in artificial containers, ground pools or slowly moving streams, can develop in abundance independently of rain water.

The frequency with which St. Louis virus isolates have been made from *C. quinquefasciatus*, together with population studies, indicate that the species is an important vector of this disease in the

Lower Rio Grande Valley of Texas. Two SLE virus isolates were made in the early fall months during the 1954 epidemic in Hidalgo County (Sullivan, *et al.*, 1957), a single isolate the following January and eight isolates in August during the 1957 outbreak in Cameron County. The findings reported here concerning isolation of SLE virus from *Culex quinquefasciatus* mosquitoes support the belief that *Culex pipiens* complex (*C. pipiens*, *C. quinquefasciatus* and *C. molestus*) are probably the principal vectors of this virus (Razenhof, *et al.*, 1957; Beadle, *et al.*, 1957; Hammon, *et al.*, 1945). *Culex tarsalis*, of special concern in western encephalitis transmission, is an uncommon mosquito in the extreme southern portion of Texas. Hammon, *et al.*, (1944) reported only 60 *C. tarsalis* in a total of 22,768 mosquitoes collected in Cameron County in 1942. The species has seldom been taken during more recent studies in the area.

Even with this information concerning the probable vector, the natural history of St. Louis encephalitis in the Rio Grande Valley of Texas is poorly understood. Studies are underway to determine which of the many species of known susceptible birds and mammals abounding in the area are actually of significance as reservoirs of the infection. Serological studies conducted during both the 1954 and 1957 outbreaks revealed that, on the basis of antibody levels, subclinical or inapparent infections greatly outnumbered the clinical cases. During these epidemics such large numbers of people have shown clinical and/or serological evidence of exposure, that the possibility of person to person transmission via *Culex* mosquitoes has been thought worthy of additional investigation.

**SUMMARY.** During investigations of the St. Louis encephalitis epidemic which occurred in Cameron County, Texas, during August of 1957, a total of 1,900 mosquitoes were collected, 85 percent of which were *Culex quinquefasciatus*. St. Louis encephalitis virus was isolated from eight pools of *Culex quinquefasciatus*. Subse-

quent to the outbreak, 3,082 mosquitoes were collected (December 1957, January, April and May 1958) and processed in 61 pools, with no virus isolates.

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