THE IMPORTANCE OF AUTUMN RAINFALL AND SENTINEL FLOCK LOCATION TO UNDERSTANDING THE EPIDEMIOLOGY OF ST. LOUIS ENCEPHALITIS VIRUS IN INDIAN RIVER COUNTY, FLORIDA[1]

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ABSTRACT. *Culex nigripalpus* adults were collected from sentinel chickens at 4 sites in Indian River Co. (IRC), Florida. Chickens located in well drained, open habitats attracted fewer *Cx. nigripalpus* than did those located in a moist, forested area, but the number of mosquitoes collected in the open habitats increased significantly with heavy autumn rainfall. All St. Louis encephalitis virus (SLE) seroconversions (11/674) during a 7-year period were from chickens located in open, well drained habitats. A comparison of monthly rainfall during years of known SLE activity in IRC with the 22-year average showed that virus activity was significantly associated with years in which rainfall was unusually low in either September or October. Sentinal chickens in areas with large vector populations may not be the most reliable indicators of local SLE activity and autumn rainfall in September and October may influence yearly SLE patterns in southern Florida.

INTRODUCTION

Domestic and wild sentinel vertebrates are often used to monitor specific areas for encephalitis virus (Nichols et al. 1975, Dickerman and Scherer 1983, McLean et al. 1983). Adult chickens are the most commonly used sentinel animals (LaMotte et al. 1967). Generally, from 2 to 24 birds are placed into secluded, all-weather, outdoor cages in areas where virus activity is suspected or known. These cages allow access to field mosquitoes which, presumably, feed freely and, if infected, transmit virus to one or more of the sentinel birds. Once a week one or more of the sentinels is bled on a rotating schedule, and the blood is checked in the laboratory for hemagglutination-inhibition (HI) antibody to specific arboviruses. An HI positive test (e.g., a rise in HI antibody titer from less than 1:10 to greater than or equal to 1:40 on a single serum sample) indicates that the bird has been bitten by an infected mosquito and that virus is present in the area. In Florida all positive samples must be confirmed by a second bleeding taken as soon as possible after the positive results of the first bleeding are known. A severe shortcoming of this technique is that confirmation of a positive HI test may come many weeks after the infective bite, thus indicating past history in terms of virus activity in the area of the sentinel animals.

In southern Florida St. Louis encephalitis virus (SLE) and eastern equine encephalitis virus (EEE) are enzootic and considered potential human pathogens. The bird-to-human vector of SLE is *Culex nigripalpus* Theobald (Chamberlain et al. 1964). The identity of the bird-to-horse or -human vector of EEE in Florida is still uncertain but *Cx. nigripalpus* may be involved (Downs et al. 1959).

Thirty Florida counties currently maintain sentinel chickens to monitor areas for SLE and EEE. Some counties maintain sentinel birds in the field all year. In Indian River County (IRC) 4 separate sites, each with 2 caged chickens, were maintained from July through November 1983. One bird from each pair was bled weekly on a rotating schedule (King 1983) to minimize the time lapse between bleeding and the final report from the Florida Department of Health and Rehabilitative Services Laboratory in Tampa where blood samples were analyzed.

Several factors determine the placement of sentinel flocks. They are placed: 1) in areas where *Culex* spp. are known to occur commonly, 2) where they are easily accessible to mosquito control personnel for maintenance and weekly bleeding, and 3) where they are secluded to reduce the chance of vandalism. The day-to-day status of vector mosquito populations is not often considered when flock sites are selected. In fact, little is known about mosquito presence and behavior at and around sentinel animals other than the indirect evidence of blood feeding or probing in the form of positive virus seroconversions. Our purpose in the present study was to use weekly aspirator collections to monitor resting adult *Cx. nigripalpus* at sentinel cages in 4 IRC locations. We were able to compare weekly mosquito catches at each flock with the local rainfall at all 4 sites. We were also able to compare SLE virus activity during the 1978–1984 seasons at each site with the number of mosquitoes caught in 1983.

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MATERIALS AND METHODS

SENTINEL FLOCKS. Indian River County is on the east coast of peninsular Florida with its center at 27°45'N, 80°35'W. In August-November 1983, pairs of sentinel chickens were maintained at 4 study sites in the county.

Site I was 6.4 km SW of Vero Beach in the center of a subtropical cabbage palm (*Sabel palmetto*)/live oak (*Quercus virginiana*) hammock surrounded by citrus groves. The ground and tree trunks in the hammock were covered with *Nephthytis liberica* of the aroid family. Site II, 9.6 km WNW of Vero Beach in a residential area, was in an open backyard. The only nearby forested area was 50 m to the east and was dominated by slash pine (*Pinus elliottii*). Daily rainfall data for sites I and II were collected by the Florida Division of Forestry at a station 3.5 km NW of site I and 6.4 km SE of site II.

Site III was 14.4 km NNW of Vero Beach in a live oak wood on an elevated sand ridge. Daily rainfall data for this site were collected by personnel at MAAG Agrochemical Inc. 3.1 km SW of the sentinel birds.

Site IV was 22.4 km NNW of Vero Beach in a dense dwarf live oak (*Quercus minima*)/sawpalmetto (*Serenoa repens*) habitat which was dry and open. Daily rainfall data for this area were collected nearby at the Florida Division of Forestry Roseland fire tower.

The number of *Cx. nigripalpus* mosquitoes in each weekly collection for each site was compared to the rainfall recorded for each site 48 hr prior to the collection period using Kendall's coefficient of rank correlation.

MOSQUITO COLLECTIONS. Resting mosquitoes in and around each sentinel cage were collected by power aspirator for 5 min per site once a week during the study period, usually on Friday mornings. The flocks were visited in the same sequence each week. Mosquitoes resting inside and immediately around the sentinel cages were collected with a hand-held aspirator 50.8 cm long, powered by a 12V battery. The aspirator had a diameter of 17.8 cm with a 43 cm long, removable net bag made to fit inside. A 12V direct current motor from Dayton Mfg. Co. ran a fan which drew air and mosquitoes through the aspirator into the bag. The live mosquitoes were returned to the laboratory, chilled, and the number of female *Cx. nigripalpus* in each collection counted and recorded.

SLE ANTIBODY IDENTIFICATION. Once a week, 3.0 ml of blood were collected from the wing vein of 1 caged chicken; thus, each chicken was bled twice a month. Blood samples were returned to the laboratory, centrifuged, and the serums mailed to the Florida Department of Health and Rehabilitative Services (HRS) Laboratory in Tampa where each was analyzed by HI test for SLE antibody. According to HRS policy any chicken which showed a four-fold increase in SLE antibody titer was bled again as soon as possible for confirmation of SLE infection.

The IRMCD sentinel chicken program began in 1978, giving us 7 years of seroconversion data for each site (Table 1). With these data we were able to use a 2 × 2 test of independence (using the G-statistic) to test for the association of habitat type and SLE seroconversions in Indian River County.

Table 1. Seven-year record of SLE seroconversions in chickens at 4 sentinel flocks in Indian River County, Florida.

	IRMCD sentinel flock number					
Year	I	II	III	IV	Total	% Positive
1978	0/24	0/24	0/24	0/23	0/95	0
1979	0/22	1/22	0/22	1/21	2/87	2.3
1980	0/33	2/33	0/33	3/33	5/132	3.8
1981	0/22	0/21	0/21	0/21	0/85	0
1982	0/20	0/20	0/20	0/19	0/79	0
1983	0/21	0/21	0/21	1/21	1/84	1.2
1984	0/28	2/28	0/28	1/28	3/112	2.7
Total	0/170	5/169	0/169	6/166	11/674	1.6

22-YEAR RAINFALL RECORD. Daily rainfall data are collected by the National Oceanic and Atmospheric Administration (NOAA) in Indian River County at Vero Beach Laboratories (27° 38'N, 80°27'W). Rainfall data from the Vero Beach site, (as well as from other sites throughout Florida) are published monthly in *Climatological Data*. A 22-year (1963–84) monthly average of August–December rainfall for the Vero Beach station was calculated from *Climatological Data* (Table 2). We were then able to compare August–December rainfall for 1978–84 at the Vero Beach station with the 22-year average to determine whether it was above or below normal. Months where rainfall exceeded the 22-year monthly mean plus the standard deviation were defined as "wet." Likewise, months where total rainfall was less than the 22-year mean minus the standard deviation were defined as "dry" (Table 2). A 2 × 2 test of independence was used to examine the association of unusually wet and unusually dry Septembers and Octobers with the number of SLE seroconversions for 1978–84.

RESULTS

MOSQUITO COLLECTIONS AT SENTINEL FLOCKS. Mosquitoes were consistently more abundant in

Table 2. Eight-year record of Indian River County, Florida, rainfall for August–December compared with 22 year (1963–84) mean rainfall and sentinel chicken SLE seroconversions.

Year	Monthly rainfall (mm)					No. IRC Seroconversions (Site)
	Aug.	Sept.	Oct.	Nov.	Dec.	
1977	148.8	201.7	56.6	81.5	169.7*	Not done (1 human-case)
1978	178.0	83.0**	81.3	119.9	132.1*	0
1979	138.2	442.5*	41.9**	99.6	37.3	2 (II and IV)
1980	31.0**	182.4	39.1**	89.4	57.9	5 (2-II, 3-IV)
1981	463.8*	197.1	78.0	78.5	20.3**	0
1982	238.8	187.7	78.5	297.4*	66.0	0
1983	230.4	81.3**	395.7*	40.1	109.7*	1 (IV)
1984	180.1	321.8*	92.4	346.7*	47.0	3 (2-II, 1-IV)
22-year mean (±SD)	164.2 (85.7)	197.7 (96.3)	146.0 (94.6)	89.9 (81.0)	59.2 (42.6)	

* Unusually wet months (> 22-year monthly mean + SD).
** Unusually dry months (< 22-year monthly mean − SD).

weekly collections from site I than from any of the other sites. The mean (± standard error) of *Cx. nigripalpus* taken in weekly collections at the 4 sites were: I − 288±48; II − 85±26; III − 42±10; and IV − 39±11.

The relationship between the number of *Cx. nigripalpus* taken at each site in weekly collections and the rainfall pattern 48 hr prior to the collection is shown in Fig. 1. There was no correlation between numbers of mosquitoes collected and rainfall at site I, which was a heavily vegetated forested area. Likewise, there was no correlation at site III, which was also a forested area. Mosquito collections were correlated with rainfall patterns at sites II ($P < 0.05$) and IV ($P < 0.001$) which were both well drained and open.

SLE ACTIVITY AT IRC SENTINEL FLOCKS, 1978–84. Sentinel chicken seroconversions for SLE virus were recorded at 2 sites (II and IV) in IRC since the surveillance program began in 1978 (Table 1). During this 7-year period 5/169 (3.0%) chickens seroconverted at site II, while 6/166 (3.6%) seroconverted at site IV. Both of these areas are open and well drained. There were no seroconversions at site I (0/170) nor site III (0/169) which are both in woodland. Between 1978–84, 11/674 (1.6%) positive SLE seroconversions were recorded from sentinel chickens in IRC. There was a highly significant ($P < 0.001$) positive association between habitat type and seroconversions during the 7-year study period with all of the seroconversions occurring in the well drained, open habitats.

INFLUENCE OF MONTHLY RAINFALL ON SLE ACTIVITY AT SENTINEL CHICKENS IN IRC. Monthly rainfall patterns during September–November appear to be important to the epidemiology of SLE virus at sentinel chicken flocks in IRC (Table 2). In 1979 an unusually rainy September followed by an unusually dry October were associated with late October and November SLE seroconversions at sites II and IV. In 1980 an unusually dry October was associated with 2 late October seroconversions at Site II and 3 at site IV. In 1983 an unusually dry September followed by an unusually wet October were associated with a late October seroconversion at site IV. In 1984 an unusually wet September followed by a normal October were associated with a late September seroconversion (site IV) and 2 early October seroconversions (site II). In 1981 and 1982 September–October rainfall was normal and no seroconversions were reported. There was a highly significant ($P < 0.001$) positive association between September and October rainfall patterns and observed seroconversion patterns. Years where rainfall patterns during these months deviated from normal were more likely to have virus activity.

DISCUSSION

The number of *Cx. nigripalpus* mosquitoes attracted to sentinel chicken flocks in southern Florida varied for the different habitats in which the birds were located. The chickens penned in well drained, open areas attracted fewer mosquitoes than those penned in moist, wooded areas. The proximity of flocks to the larval habitat of *Cx. nigripalpus* is also an important consideration. Sites I, II, and III were close to ditched citrus groves where *Cx. nigripalpus* commonly oviposited after heavy rains. Site IV was located several kilometers from suitable *Cx. nigripalpus* oviposition sites.

Even though fewer mosquitoes were collected at sites II and IV than at site I, all of the 1978–84 SLE virus activity in IRC was recorded in these 2 open, well drained habitats. Fur-

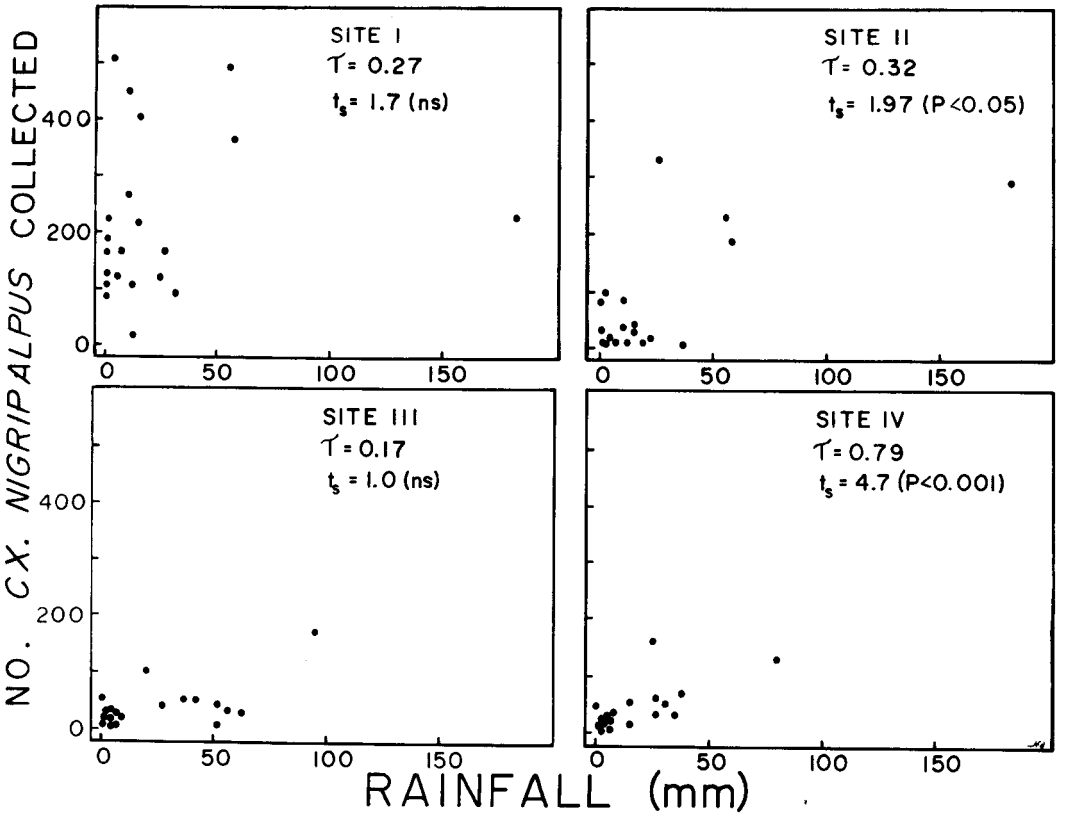

Fig. 1. Plots showing the relationship between the number of mosquitoes captured and rainfall 48 hours prior to collection for IRC sites I–IV. Significance was tested using Kendall's coefficient of rank correlation. The t_s values and level of significance are shown for each site (ns = not significant).

thermore, a significant association between the number of mosquitoes collected at sites II and IV and recent rainfall at those sites indicates that mosquitoes depend on the presence of rain and the resulting ground moisture to reach birds located in these open areas. Edman (1974) suggested that autumn rain may be one cause of the observed feeding shift of *Cx. nigripalpus* from birds to mammals (Edman and Taylor 1968). These autumn rains may allow the mosquitoes to penetrate previously inhospitable, dry habitats. We suggest that heavy autumn rainfalls enable mosquitoes to reach penned hosts located in habitats which remain inaccessible to host-seeking vectors during dry periods of the year.

The significant association between open, well drained habitat types, low mosquito populations, and positive SLE virus activity is surprising but not unique to IRC. Similar observations have been made in Sarasota County (personal communication, Ron Winner, Sarasota County Mosquito Control) and Martin County (personal communication, Lynn Erickson, Martin County Department of Environmental Services). There are several possible explanations for these observations: 1) older mosquitoes may be found in disproportionate numbers in these open habitats. 2) After a rainfall, gravid mosquitoes may fly into open areas in search of an oviposition site or, after ovipositing, may venture far to look for a host. 3) These well drained, open sites may have an associated SLE focus.

The reasons for sporadic SLE activity in Florida are not clear. There are years like 1977 during which a widespread outbreak occurred in central Florida. Associated with this outbreak were 110 confirmed and presumptive human cases and 8 deaths (Chamberlain 1980). In 1978 there was no SLE activity in Florida. We found SLE activity at sentinel flocks in IRC to be significantly ($P < 0.001$) associated with unusual autumn rainfall patterns (Table 2). In general, when September or October were significantly drier than normal, SLE activity was recorded. Provost (1969) suggested that gravid *Cx, nigripalpus* females may hold their eggs during dry

periods and thus increase the probability that, when infected, the extrinsic incubation period would be completed before a second blood meal was taken. Our observations support this hypothesis. In IRC, years where September or October were unusually dry were more likely to be years with virus activity. During these dry periods (which lasted as long as 25 days) gravid *Cx. nigripalpus* may rest and wait for suitable flying conditions (Dow and Gerrish 1970, Provost 1974) or may simply not be able to find suitable oviposition sites. If infected with virus by a recent blood meal, these mosquitoes caught by unfavorable weather conditions would have plenty of time for the extrinsic incubation of the virus. Once rains create suitable oviposition sites, the mosquitoes would lay their eggs and then host-seek as infective individuals.

Culex nigripalpus is both an enzootic and an epidemic vector of SLE in Florida (Chamberlain et al. 1964, Bond et al. 1965). Virus activity in sentinel flocks is observed most commonly during September–November (Yeller 1978, Nelson et al. 1983). Human SLE cases in Florida usually occur in October or November. We found that in IRC SLE activity is significantly greater in sentinel chickens located in well drained, open habitats where mosquito presence apparently depends upon autumn rainfall patterns. We also show that SLE activity is significantly associated with years where either September or October is unusually dry. Dry periods during these months may serve to concentrate gravid vector mosquitoes which, after suitable rainfall, ovipost and again host-seek. Likewise, after heavy rains parous mosquitoes may be stimulated to long flights into drier areas seeking hosts.

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