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ARBOVIRUS SURVEILLANCE IN RHODE ISLAND: ASSESSING POTENTIAL ECOLOGIC AND CLIMATIC CORRELATES

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ABSTRACT. During 1995–2000, mosquitoes were collected from sites throughout Rhode Island and tested for the presence of arboviruses. Mosquito trapping was done weekly from June to October with CO_2-baited light traps. In all, 186,537 mosquitoes belonging to 7 different genera were collected, of which *Coquillettidia perturbans* was most abundant. A total of 6,434 pools were processed for arbovirus isolation, from which 193 arboviral isolations were made. These included 109 Highlands J, 71 eastern equine encephalomyelitis, 1 California encephalitis serogroup, 2 Jamestown Canyon, 3 Cache Valley, and 9 Flanders viruses. Our isolations of Flanders virus represent the 1st reported occurrence of this virus in Rhode Island. After the 1999 sudden occurrence of the West Nile virus (WN) in the New York City area, a dead-bird surveillance program was started to test for this virus. Although no isolations of WN were made from mosquitoes, 87 virus isolations were made from a total of 330 wild birds tested. All the WN-infected birds were either American crows or blue jays. Isolation of WN from dead birds marked the 1st documented appearance of this virus in Rhode Island. Significant interannual variation of arbovirus activity in Rhode Island prompted us to examine if climate-associated factors such as rainfall and temperature correlate with virus activity. Total rainfall amounts from May to June were higher than normal in 1996 and 1998. These years showed significantly higher arbovirus activity. Deviations from normal temperature showed low correlation with arbovirus activity during the 6-year study period. Therefore, precipitation appeared to be more important than temperature in predicting arbovirus activity in Rhode Island.

KEY WORDS Arbovirus activity, climate, mosquitoes, precipitation, Rhode Island

INTRODUCTION

Knowledge of arbovirus activity in Rhode Island is limited to mosquito surveillance results, and the relation of arboviral activity with environmental or ecologic factors are poorly understood. Human disease caused by mosquito-borne viruses is relatively rare in the northeastern USA. However, when they occur, human infections lead to significant morbidity and mortality. Of the arboviruses known to cause disease in Rhode Island, eastern equine encephalomyelitis virus (EEE) is probably the most important (Gettman 1993, Markowski 1996). Sporadic epizootics of EEE have occurred in Rhode Island horse and bird populations since 1938. In total, more than 100 horse deaths attributed to EEE have been reported, and several thousand game birds have died as a result of EEE infection. In Rhode Island, 4 human cases caused by EEE have been confirmed since 1983. The 1st 2 cases occurred in 1983, resulting in 1 fatality (Centers for Disease Control and Prevention [CDC], 1984). In 1993, a 14-year-old boy was diagnosed with infection by EEE and died a few weeks later (Bandy and Donnelly 1994, Sotomayor and Josephson 1999), and in 1998 an elderly man also died from EEE infection (RIDOH, unpublished data). Two emus died of EEE infection in the southern part of Rhode Island in 1996. An arbovirus surveillance program began in 1984 as a direct result of the 1st human cases in 1983. Eastern equine encephalomyelitis virus was isolated for the 1st time in Rhode Island from *Culiseta* and *Aedes* mosquito species in 1990 (Cookman, unpublished data). In addition to EEE, surveillance identified a number of other arboviruses, including Highlands J (HJ), Jamestown Canyon (JC), Cache Valley (CV), and Flanders viruses from several mosquito species in Rhode Island.

During late summer and early fall of 1999, an episode of West Nile virus (WN) occurred in the New York City area, resulting in fatal neurologic disease in humans and a variety of native and exotic bird species (CDC 1999a, 1999b, 1999c). That occurrence resulted in 62 human cases, including 7 deaths and thousands of bird deaths. In 2000, the epizootic spread throughout the northeastern USA, including Rhode Island, but few human infections were identified (CDC 2000). Phylogenetic analysis of the New York strain of WN (WN-NY99) has shown it to be most closely related to a strain of WN isolated from a goose in Israel in 1998 (Lanciotti et al. 1999). Although WN is common in parts of Africa, western Asia, and the Middle East, the New York episode was the 1st incidence of this virus reported in the Western Hemisphere. The virus is now well established in North America, with

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isolations occurring as far north as Canada and as far south as Florida.

Increasingly, attention is being focused on the relation between climate variation and vector-borne diseases (Longstreth and Wiseman 1989). The recent El Niño and La Niña events and the prospect of global warming have stimulated interest in developing predictive models to help forecast the onset of vector-borne disease epidemics (Reeves et al. 1994, Hales et al. 1999, Maelzer et al. 1999). For example, several studies in the USA have focused on associations between temperature and rainfall amounts and infections with EEE. In 1 of those studies, Hayes and Hess (1964) found a relation between precipitation and human EEE cases only when excessive rainfall and the cases occurred in the same summer and in the fall of the year preceding the epidemic. In Massachusetts, epidemics caused by EEE occurred in the 2nd of successive years when rainfall was 20 cm above average (Grady et al. 1987). More recently, Letson et al. (1993) reexamined the hypothesis that excessive rainfall is predictive of the occurrence of human disease cases caused by EEE. They found an association between the occurrence of human cases and excessive rainfall and that the association was stronger with data from local weather stations than from statewide rainfall averages. Moreover, these models were most predictive when applied to the northern states. Unusually heavy rainfall has been implicated as a possible factor contributing to previous occurrences of WN in South Africa (Jupp 2001), but dry conditions existed during the summers when WN episodes occurred in southern Romania, Russia, and the northeastern USA (Hayes 2001).

This report summarizes mosquito-borne virus activity in Rhode Island during the 6-year period, 1995–2000, and examines relations between virus activity and climate factors.

MATERIALS AND METHODS

Mosquito collections: Weekly mosquito collections were made in Rhode Island from June to October, 1995–2000, with CDC light traps baited with dry ice. Mosquito collections were immediately placed on dry ice for transport to the laboratory, where they were stored at −80°C until processed. All mosquitoes were identified on a chill table, and females were sorted into pools according to species, site, and collection date. The number of mosquitoes was ≤50 per pool.

Bird collections: After the WN occurrence of 1999 in New York, a dead-bird surveillance program was established to facilitate virus detection in Rhode Island. Dead birds were collected statewide by the Rhode Island Department of Environmental Management and transported to the Rhode Island Department of Health Laboratories for brain necropsy. The brains were placed in dry ice and shipped to the BSL-3 laboratory at the Center for Vector-Borne Disease, University of Rhode Island, for virus isolation and identification.

Virus assays: Adult mosquitoes collected from 1995 to 1997 were screened for viruses at the Arbovirus Research Laboratory at Yale University. In 1998, mosquito pools were tested by the Massachusetts Department of Public Health. In 1999, mosquito testing was permanently transferred to the Center for Vector-Borne Disease at the University of Rhode Island.

In all 6 years, mosquitoes were tested by the same procedure. Briefly, mosquito pools were homogenized in the presence of 199 medium containing 1% fetal calf serum and 500 IU/ml penicillin and 500 μg/ml streptomycin. Homogenates were centrifuged at 3200 × *g* for 20 min at 4°C to pellet particulate material. Aliquots (0.1-ml) of each supernatant were inoculated onto a monolayer of Vero cells growing in 6-well plates and incubated at 37°C under 5% CO_2 for up to 7 days. Cultures were examined daily for cytopathic effect.

During 1999–2000, specimens were also tested by plaque assay in a Vero cell culture growing in 6-well plates. Briefly, specimens were inoculated in 0.1-ml quantities and adsorbed for 1 h at 37°C, then the cells were overlaid with 2 ml of a mixture containing 1% agar and 0.1% DEAE in 199 medium. Cultures were incubated for 48 h, and a secondary overlay containing 10% neutral red and 1% agar in 199 medium was added to the wells. Cultures were incubated at 37°C and examined for up to 7 days for the presence of plaques.

Supernatants from positive cell cultures were harvested and frozen at −80°C, and cells were scraped from the wells to prepare a cell lysate antigen (Ansari et al. 1993). Viruses were identified by indirect immunofluorescent antibodies (IFA) by using reference monoclonal antibodies provided by the Division of Vector-Borne Infectious Diseases, CDC in Fort Collins, CO. These included EEE, HJ, western equine encephalomyelitis virus, LaCrosse virus (LAC), WN, and St. Louis encephalitis virus (SLE) antibodies. Dr. Robert Shope, University of Texas Medical Branch, Galveston, TX, provided reference antibodies to JC and Flanders viruses. Twelve-well slides (Cel-Line Associates, Inc., Newfield, NJ) containing virus-infected cells were fixed in acetone, incubated with monoclonal antibodies for 1 h at 37°C, and then washed 3 times with phosphate buffered saline. Slides were then incubated with fluorescein isothiocyanate–labeled goat anti-mouse IgG (1:200 dilution; Kirkegaard and Perry Laboratories, Inc., Gaithersburg, MD) for 1 h at 37°C and examined under a fluorescence microscope for a positive reaction.

Positive samples from 2000 were also confirmed by reverse transcriptase polymerase chain reaction (RT-PCR) of the cell culture supernatants and directly from avian brain tissue by virus-specific primers for EEE (Armstrong et al. 1995), HJ

Table 1. Summary of mosquitoes collected and arboviruses detected in Rhode Island, 1995–2000.

Year	Total no. of mosquitoes		No. of pools tested	No. of positive pools	% of pools positive	Individual viruses				
	Collected	Tested				EEE	HJ	CE/JC	WN	Other
1995	20,389	16,461	822	10	1.2	0	6	1	NT[1]	3
1996	45,007	35,957	1514	100	6.6	56	43	1	NT	0
1997	46,266	27,814	1087	18	1.7	2	16	0	NT	0
1998	35,240	18,539	776	49[2]	6.3	12	39	0	0	0
1999	27,722	19,065	1114	3	0.3	0	0	1	0	2
2000	11,949	10,688	1121	13	1.2	1	5	0	0	7
Total	186,537	128,524	6434	193	3.0	71	109	3	0	12

[1] NT, not tested.
[2] Two pools were co-infected with EEE and HJ.

(Whitehouse et al. 2001), and WN (Lanciotti et al. 2000).

Climatological data: Precipitation and temperature data were taken from 13 National Weather Service (NWS) weather stations in Rhode Island and nearby Connecticut and Massachusetts, with supplemental information added from the Army Corp of Engineers (ACE) stations in the same general area. The NWS stations provide average monthly climate variables and climatic information at time increments as frequent as hourly. The ACE data were included to provide increased spatial resolution but were often available only as a monthly summary. Combining these sources provided a dense climate observation network from which to document the dominant weather patterns statewide for any particular month as well as for the mosquito season. Temperature data were analyzed based on monthly as well as seasonal averages. Average monthly temperature and departure from the 30-year normal monthly temperature were computed for 1990–99. Precipitation was examined for monthly and multi-month accumulations and for any duration of insufficient precipitation. Deviations from 30-year monthly normal precipitation amounts were computed.

Statistical analyses: Simple linear regression was used as the primary statistical method to evaluate relations between climate and mosquito-borne virus activity. The annual number of virus-positive mosquito pools for each virus was correlated with precipitation and temperature during the time periods previously identified. Chi-square and Student's *t*-tests were used to analyze annual differences in virus activity. All statistical tests were performed using SPSS v7.5 for Windows.

RESULTS

Mosquito surveillance: A total of 187,537 mosquitoes were collected and identified in Rhode Island between 1995 and 2000 (Table 1). Of these, 128,524 were tested for virus in a total of 6,434 pools. *Coquillettidia perturbans* (Walker) was the most abundant mosquito collected (21% of the 6-year total). During the 6-year study period, 193 mosquito pools were positive for arboviruses, representing 3% of the total pools tested. The most prevalent arbovirus isolated was HJ (109 isolates, comprising 56.5% of the total). Eastern equine encephalomyelitis virus was isolated 71 times (36.8% of total isolations), making it the 2nd most commonly detected virus. Other virus isolations included California Encephalitis serogroup (2), JC (1), CV (3), and Flanders (9) (Fig 1). Isolation of Flanders virus in 1999 represented the 1st appearance of this virus in Rhode Island. There were no isolations of either LAC or SLE during the study period. Also, there were no isolations of WN from mosquitoes between 1995 and 2000, although numerous isolations were obtained from wild birds in 2000 (see below). Retrospective testing of mosquito pools from 1998 and 1999 failed to detect WN-positive samples.

Mosquitoes collected during 2 of the 6 study years had significantly higher levels of virus activity ($P < 0.05$) than the other years. In 1996 and 1998, the percentages of mosquito pools positive for virus were 6.6 and 6.3%, respectively, whereas virus-positive rates for mosquito pools tested during the other years never exceeded 1.7%. Except for single isolations of EEE in North Smithfield and HJ in Lincoln, all viral isolations were from mosquitoes collected in southern Rhode Island (Washington County), where 53% of the trapping effort occurred. The majority of isolations were made in the southwestern part of the state, especially in or near the town of Westerly. This area has an abundance of mixed hardwood swamps and coastal salt marshes, which may contribute to larger populations of mosquitoes carrying these viruses.

Dead-bird surveillance: After the WN episode in New York City in the late summer and early fall of 1999, Rhode Island and many other eastern states began a dead-bird surveillance program for detecting WN. A total of 330 wild birds were tested for the presence of virus activity during 2000 (Table 2). Of those, 95 (27.3%) were positive. The majority was infected with WN (26.4%); WN was 1st isolated in Rhode Island from an infected crow

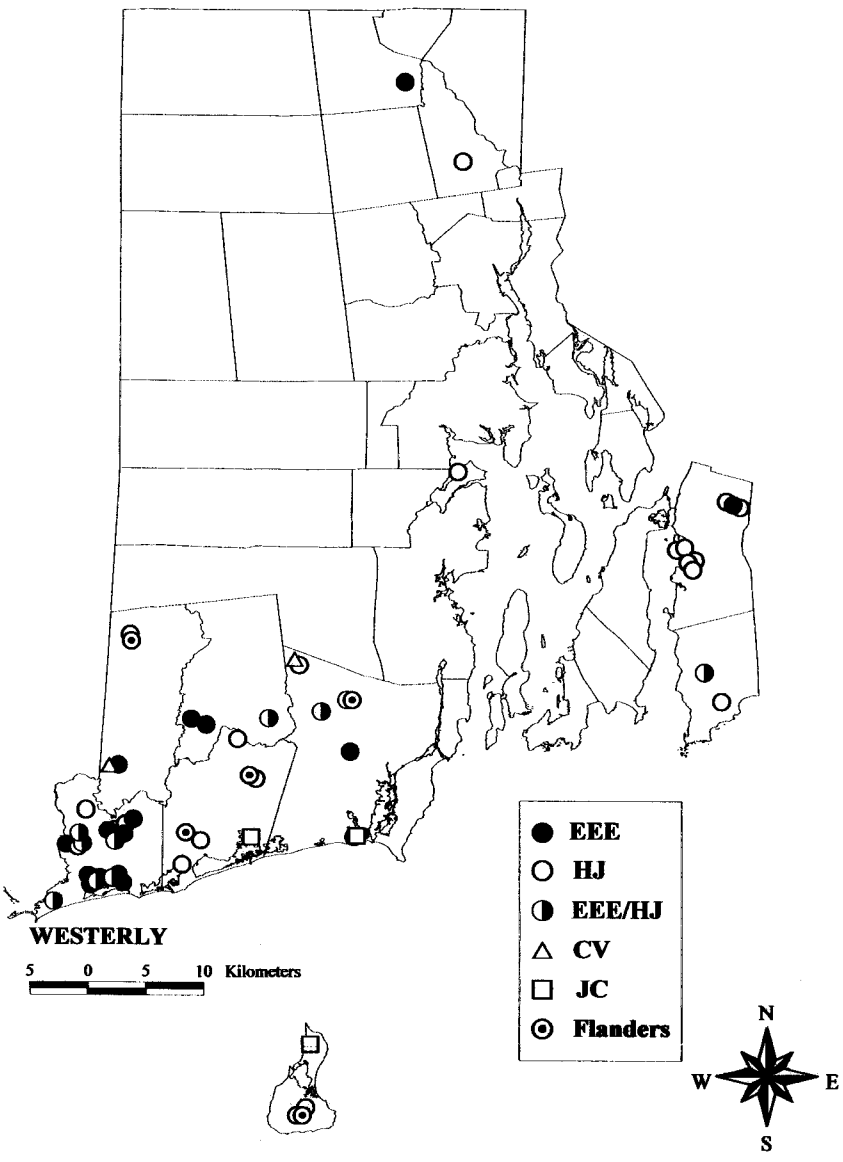

Fig. 1. Map of Rhode Island indicating the towns and locations where arboviral isolations were made from collected mosquitoes. EEE, eastern equine encephalomyelitis; HJ, Highlands J; CV, Cache Valley; JC, Jamestown Canyon.

on August 8, 2000. Figure 2 shows collection sites of WN-positive birds. Other viral isolations from dead birds included 4 EEE (1.2%) and 2 HJ (0.6%). Additionally, 2 of 15 pigeons tested were infected with Rhode Island virus, a newly described rhabdovirus (Travassos da Rosa et al. 2002). Of the WN-infected birds, American crows (*Corvus brachyrhynchos*) were infected most commonly (79.3%, $n = 69$), whereas blue jays (*Cyanocitta cristata*) (20.7%, $n = 18$) were the only other species infected with WN. The EEE isolations were made from a variety of birds including American crow, blue jay, house sparrow (*Passer domesticus*), and a northern raven (*Corvus corax*). Highlands J was the only virus isolated from sparrows (Whitehouse et al. 2001). The WN positivity rate among birds increased through late summer and fall with 4.6% positive in August, 37.9% in September, 43.7% in October, and 13.8% in November. The weekly positive rate among crows tested peaked during the 2nd week of October and began to fall with the onset of colder weather (Fig. 3). A cluster of 39 WN-infected birds (44.8% of total WN-positive birds) was detected in the town of Westerly, RI (Fig. 2). The significance of this clustering is not yet known but may be related to the finding and reporting of dead birds in Rhode Island communities.

Table 2. Arbovirus isolations from wild birds collected in Rhode Island during 2000.

Virus[1]	Bird species	No. tested	No. positive	% positive
WN	American crow	134	69	49.5
	blue jay	48	18	37.8
EEE	American crow	134	1	0.9
	bluejay	48	1	2.2
	sparrow	30	1	3.7
HJ	northern raven	1	1	100
	sparrow	30	2	7.4
Total		330	95[2]	27.3

[1] WN, West Nile virus; EEE, eastern equine encephalomyelitis; HJ, Highlands J.

[2] Includes 2 pigeons that were infected with an unknown rhabdovirus isolate.

Climatological factors: Significant interannual variation of virus activity prompted us to examine climate variables (e.g., temperature and rainfall) for possible correlation. The May–July total rainfall amounts were higher in 1996 (29.8 cm) and 1998 (50.6 cm), years with significantly higher virus activity. For example, rainfall in Rhode Island during this period in 1998 was 27.5 cm higher than the 30-year average for the region (Fig. 4a). The positive correlation of rainfall with mosquito pool virus positivity was highest with HJ (0.86; Table 3). Eastern equine encephalomyelitis virus showed the lowest correlation with rainfall with a maximum of 0.38. Correlations were negative but high at −0.91 for mosquito-borne viruses other than HJ and EEE.

Comparing the monthly mean temperatures with the Rhode Island 30-year average, we observed warmer-than-normal temperatures during the later half of the 1990s. These data were consistent with those from other USA stations (Fig. 4b). Deviation from normal temperature showed the highest correlation of any temperature measurement to virus activity during the 6-year study period. However, correlation still tended to be low (Table 3). Correlations ranged between negative and positive values depending on the virus, reaching a maximum of −0.69 for "other viruses." The ratio of EEE/HJ was positively correlated with temperature but reached a maximum of just 0.76.

DISCUSSION

Mosquito surveillance for arbovirus activity conducted in Rhode Island during the 6-year period, 1995–2000, was compared with measures of precipitation and ambient temperature. Of the viruses isolated from 193 positive mosquito pools, HJ was the most prevalent, followed by EEE. The majority of isolates were from *Culiseta melanura* (Coquillett), an ornithophilic mosquito incriminated as the primary enzootic vector of both EEE (Scott and Weaver 1989, Morris 1988) and HJ (Hayes and Wallis 1977). In some years (e.g., 1996), EEE also was isolated from a number of other mosquito species including *Ochlerotatus canadensis* (Theobald), *Aedes vexans* (Meigen), and *Cq. perturbans.* During 1996, a significantly higher level of EEE activity in Rhode Island was identified, which most likely resulted in a spillover of EEE from its primary enzootic vector into a number of other, potentially epizootic vector species. Indeed, during 1996, 2 fatal infections of EEE were reported in emus in southern Rhode Island. In southeastern Connecticut, Andreadis et al. (1998) reported 36 isolations of EEE from 8 different species of mosquitoes. Eastern equine encephalomyelitis virus is considered to be the most important mosquito-borne arbovirus occurring in Rhode Island, causing sporadic cases of disease among horse and bird populations.

Examining isolations of EEE from mosquitoes during the 6-year study period revealed a pattern of interannual variation in which 1996 and 1998 had significantly higher levels of EEE than the other years. These "high-virus" years corresponded to sporadic cases reported in emus in 1996 and a fatal human case in 1998. In 2000, the only year for which we have data on EEE in native birds, the virus was isolated from the brains of 4 different species, including an American crow, blue jay, sparrow, and northern raven. These birds showed no obvious signs of trauma, and the virus was isolated directly from brain tissue; therefore, we speculated that these native bird species died as a result of infection with EEE.

The enzootic transmission cycle of HJ is similar to EEE, being transmitted by *Cs. melanura.* among passerine birds in freshwater swamps. Moreover, like EEE, HJ exhibited peak prevalence in Rhode Island in 1996 and 1998. However, unlike EEE, HJ has not been shown to be pathogenic in humans or horses, with the exception of a single report of the virus being isolated from a horse that died of encephalitis in Florida (Karabatsos et al. 1988). Highlands J is emerging as an important bird pathogen, causing disease in a number of domestic avian species (Ficken et al. 1993, Eleazer and Hill 1994). Apparently, HJ also may be pathogenic for native birds, as the virus was recovered from the brains of 2 dead sparrows in 2000 (Whitehouse et al. 2001). Wild birds such as sparrows usually are considered reservoirs for many mosquito-borne arboviruses and are not normally expected to succumb to infection with these viruses. The relatively low numbers of wild birds infected with and presumably killed by EEE and HJ (as compared with WN-infected birds) do indicate that the 2 sparrows may have had some undetected preexisting infection or had been exposed to an environmental stress that weakened their immune systems, thereby making them more vulnerable to any pathologic effects of the HJ infection. We cannot rule out the possibility of increased virulence or neuroinvasiveness of specific EEE or HJ strains. Additional isolations and

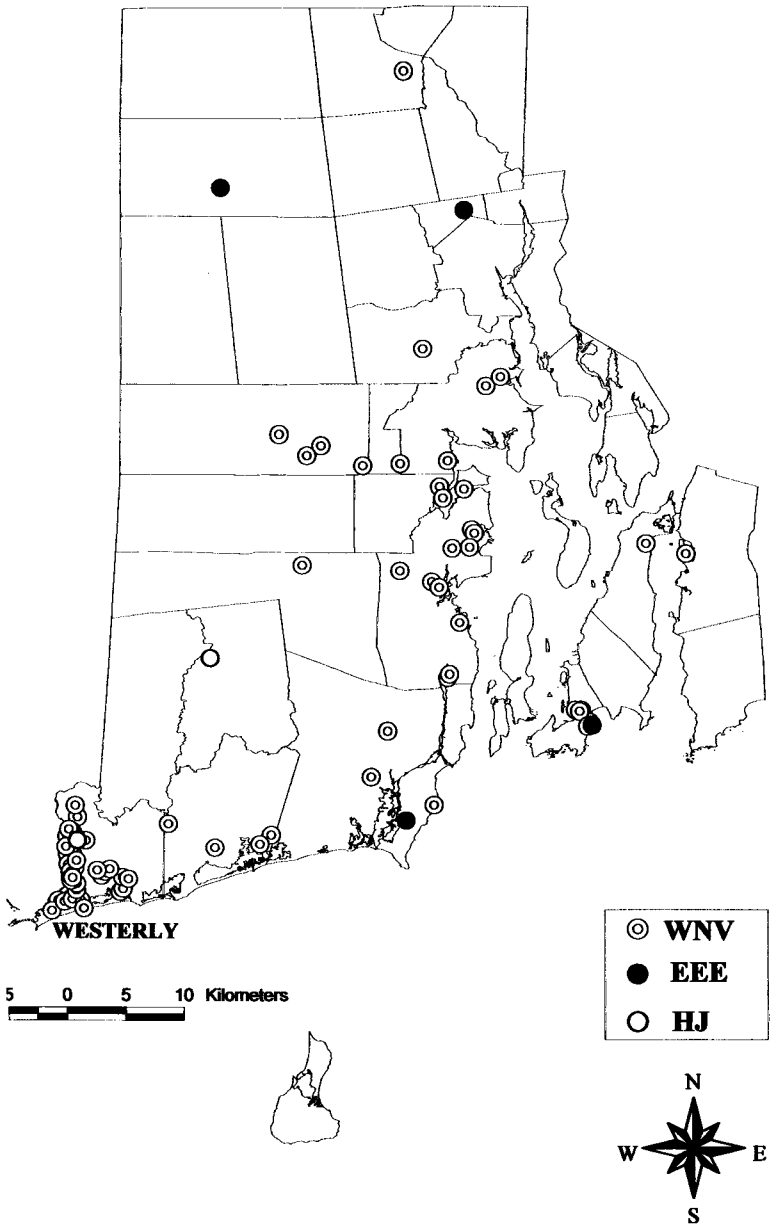

Fig. 2. Map of Rhode Island indicating the towns and sites where West Nile virus-infected birds were collected. WNV, West Nile virus; EEE, eastern equine encephalomyelitis; HJ, Highlands J.

genotyping from bird surveillance programs will be needed to answer such possibilities.

The 1st isolation of Flanders virus in Rhode Island was made in 1999 from a pool of *Culiseta* mosquitoes collected from the southwest part of the state. During 2000, the virus was isolated from 7 additional mosquito pools collected from the same area. Flanders virus was 1st isolated from a pool of *Cs. melanura* mosquitoes collected in 1961 in Flanders, Long Island, NY (Whitney 1964). Flanders virus is not known to cause disease in vertebrates and produces very small plaques in tissue culture, thus it may have been overlooked previously in Rhode Island.

Isolations of CV in 1995 and JC in 1996 and 1999 confirm the presence of these California serogroup viruses in Rhode Island. Only 2 isolations of JC were made during the study period, both from *Oc. cantator*. Jamestown Canyon also has been identified from the neighboring states of Connecticut and Massachusetts (Sprance et al. 1978, Walker et al. 1993). Therefore, it was not surprising that

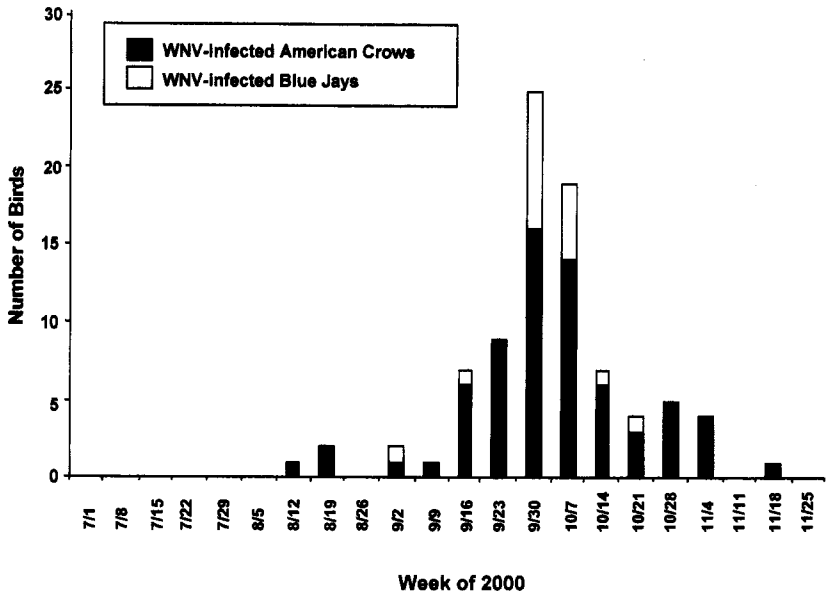

Fig. 3. Weekly infection rates of West Nile virus-infected American crows and blue jays collected in Rhode Island during 2000.

the virus was isolated from Rhode Island mosquitoes as well. Three isolations of CV from *Oc. canadensis* were identified, and an untyped California serogroup virus was isolated from *Cq. perturbans* in 1995, but no additional isolations of CV have been made in Rhode Island. Cache Valley virus has been shown to cause embryonic death and congenital malformations of sheep (Edwards et al. 1989), and retrospective serologic studies in humans suggest that CV may be the cause of some cases of congenital defects of the central nervous system (Calisher and Sever 1995). Recently, the 1st human case of severe encephalitis and multiorgan failure caused by CV virus was described in a patient from North Carolina (Sexton et al. 1997). The geographic distribution of this virus includes all of North America, except the extreme southeastern states and southern Mexico (Calisher et al. 1986). Prevalence of the virus in Rhode Island appears to be rare.

The sudden occurrence of WN in the New York City area in 1999 prompted many states in the eastern USA to increase their mosquito-borne arbovirus surveillance efforts beginning in 2000. The sensitivity of birds, especially crows, to WN also prompted many states, including Rhode Island, to adopt a dead-bird surveillance program to aid in the early detection of WN. Of the 330 dead birds tested during 2000, 87 (26%) were infected with WN as determined by direct culture and RT-PCR. The American crow was by far the most common species infected, although blue jays also were infected. Dead-bird surveillance was very effective as an early warning system for WN; 87 isolations of the virus were made from birds, but no WN-infected mosquitoes or human cases were detected. The effectiveness of bird surveillance for predicting human or horse WN infection remains unknown. It appears that bird surveillance data may well depict the geographic range of the virus but not accurately reflect human risk for infection. However, mammal-biting mosquitoes infected with WN must have been present in the state because a 2-year-old horse from Wakefield, RI, with no history of travel outside the state became infected with WN and was euthanized.

In 2000, our laboratory began using RT-PCR analysis in addition to traditional biological assays for detecting arboviruses. This molecular-based assay offers the advantage of being more rapid and more specific than classical culturing techniques for detecting viruses. The RT-PCR assay correctly identified every EEE, HJ, and WN isolated in the 2000 season (data not shown). Results were 100% concordant with the biological assay of isolation in Vero cell culture and identification by IFA with reference monoclonal antibodies. We were not able to obtain a positive PCR product of Flanders virus but were able to identify it by IFA.

The mosquito vectors of various arboviruses likely respond differently to climatic variation. Accordingly, we examined possible relations between arbovirus isolations and climate factors in Rhode Island. As in several other studies, we found links between precipitation and virus activity (Hayes and Hess 1964, Grady et al. 1987, Letson et al. 1993). However, the correlation between EEE activity in mosquitoes and precipitation was insignificant, regardless of the time period measured. Highlands J activity showed maximum positive correlation

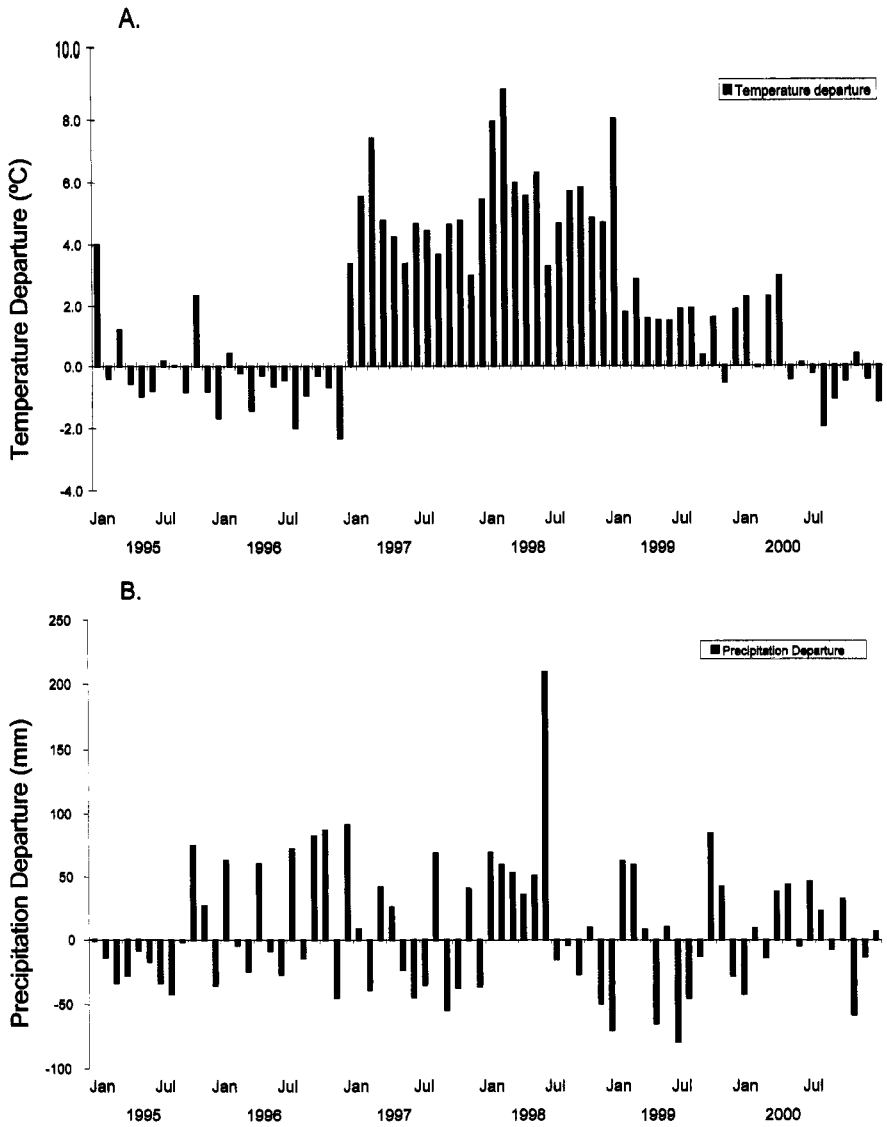

Fig. 4. Departure from a 30-year average (1961–1990) of the mean monthly (A) temperature and (B) precipitation in Rhode Island during 1995–2000.

Table 3. Results of linear regression correlating climate factors to annual positive mosquito pools.[1]

Time period	Climate factor	Correlation values[2]			
		EEE	EEE/HJ	HJ	Others[3]
Jan–May	Precipitation	0.3083	0.6121	0.6032	−0.8841[4]
	Temperature	−0.3703	0.7573[5]	0.2294	−0.5839
Jun–Aug	Precipitation	0.3838	0.8320[4]	0.8576[4]	−0.7855[5]
	Temperature	−0.3605	0.6205	0.1761	−0.6900[6]
Jan–Aug	Precipitation	0.3506	0.6888[6]	0.7108[6]	−0.9105[4]
	Temperature	−0.3699	0.7155[6]	0.2126	−0.6257

[1] EEE, eastern equine encephalomyelitis; HJ, Highlands J.
[2] Significant only at levels above $\alpha = 0.15$, unless otherwise marked.
[3] California serogroup, Jamestown Canyon (JC), Cache Valley and Flanders. JC was included in the California serogroup and was detected separately.
[4] Significant at $\alpha = 0.05$ level.
[5] Significant at $\alpha = 0.10$ level.
[6] Significant at $\alpha = 0.15$ level.

Table 4. Expected variation around seasonal temperature and precipitation normals in the northeastern USA as a result of the El Niño/Southern Oscillation cycle.

	Precipitation		Temperature	
Season	El Niño	La Niña	El Niño	La Niña
Winter	Above	Above	Above	Above
Spring	Below	Normal	Normal	Normal/below
Summer	Normal/below	Normal/above	Normal/below	Below
Autumn	Below	Normal/above	Normal/above	Normal/below

(0.86) with June–August precipitation totals ($P < 0.05$), indicating an increase in activity with abundant precipitation. This also proved to be the only precipitation period with any significant correlation to virus activity ($P < 0.05$). Low-level, nonsignificant ($\alpha = 0.05$) positive correlations (0.31–0.38) of EEE with precipitation may indicate a response to how frequently the precipitation falls but not to total amounts of precipitation. No correlation of virus activity was found with either January–May (preseason) or January–October (through-season) values. Viruses labeled "Others" in Table 3, including California serogroup, CV, JC, and Flanders, showed negative correlation with precipitation, perhaps indicating a preference for drier conditions. The best correlation for this group of viruses was −0.91 with the January–August precipitation ($P < 0.05$). This group, not surprisingly, also was correlated with the January–May precipitation (−0.88, $P < 0.05$).

Temperature showed low to moderate correlation with arbovirus activity. Eastern equine encephalomyelitis virus activity tended to be lower during seasons and years with warmer-than-normal temperatures, whereas the reverse trend was observed for HJ virus activity (Table 3). However, temperature was only significantly correlated ($\alpha = 0.05$ level) with the ratio of EEE/HJ virus activity.

The significant El Niño event beginning in 1997 and ending early in 1998 has been ranked as one of the largest such events of the past century (Wolter and Timlin 1998) and has brought considerable attention to the potential impact of extreme climate variability on vector-borne diseases (Patz et al. 2000). However, the effect of El Niño on mosquito populations in the USA has yet to be assessed. In Rhode Island, El Niño contributed to lower-than-normal annual and summer-season precipitation and higher-than-normal annual and summer temperatures between 1997 and 1998 (Table 4). During the 1997 season, arbovirus activity was rare in Rhode Island. Nearly twice as many virus-positive mosquito pools were found in 1996 and 1998, years not dominated by extreme weather events. Although more observations are needed, it may be possible to project decreased virus activity in the northeastern USA related to El Niño episodes.

La Niña events represent the opposite extreme of the El Niño/Southern Oscillation (ENSO) cycle and often closely follow El Niño episodes. The La Niña event that began in mid to late 1998 has been the longest running La Niña recorded; it was still active just before the mosquito season of 2001. In Rhode Island, this La Niña likely contributed to annual and summer-season precipitation varying from well above normal in 1998 to near normal in 1999 and 2000. Additionally, warmer-than-normal annual temperatures dominated the region during 1998 and 1999, whereas near-normal temperatures were recorded during 2000. Summer season temperatures also were well above normal in 1998 but were near normal in 1999 and 2000. Although El Niño and La Niña events may not be causative in triggering changes in mosquito and virus activity in Rhode Island during the past 6 years, anomalous weather can contribute to environmental changes affecting mosquito populations and virus infection rates. It may be possible to use increasingly sophisticated dynamic and statistical models that predict episodes of climate variability, particularly ENSO, in developing models predicting increased mosquito virus activity.

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