
Arbovirus Surveillance: 2000

Tampa Branch Laboratory

This was a quiet year for arbovirus activity in Florida, probably relating to the extreme drought in much of the state. However, interest in Arbovirology increased nationwide as has testing to assure the absence of virus activity. The sentinel chicken program is a cost effective way to stay on top of the situation in Florida. Last year 27 counties participated in this project.

Mosquito control agencies or County Public Health Departments within participating counties maintained small flocks of chickens at various sites in their area. The birds were bled at specified intervals, and the samples sent to our laboratory. All specimens received in the lab by 12 noon on Wednesday were processed and assayed in that week's test. The hemagglutination inhibition test (HAI) was used to ascertain the presence of antibody to St. Louis Encephalitis (SLE) and Eastern Equine Encephalitis (EEE) viruses. Reports were expeditiously faxed to the submitters on Friday afternoon. Summary reports were also compiled and e-mailed to the County Health Department Directors for all participating counties.

Because of the emergence of West Nile virus (WN), an arbovirus new to the Americas, in the northeast and its apparent migration down the east coast, surveillance efforts have been intensified. Funding was through a supplemental ELC grant from the CDC. West Nile virus (WN) is a flavivirus, closely related to SLE. The HAI test used in this laboratory is a broadly reactive screening test, and will detect antibodies developed to WN, as well as to SLE. Thus, our surveillance program is capable of detecting the presence of both of these arboviruses. Nevertheless, to ascertain which virus is present requires additional testing. The IgM Elisa assay provides a means to distinguish between the two with sera from humans or chickens, but not other animals. However, some cross-reactions may occur in this assay. All SLE HAI positive sentinel sera from this season with sufficient residual available, were assayed by the "Chicken Elisa" protocol as developed by CDC. No West Nile antibody positive birds were detected that were not also strong reactors for SLE IgM.

The most specific test to distinguish between antibodies developed to these viruses is the serum neutralization assay. Any type of serum (mammal, bird, reptile, etc.) can be tested by this assay. This quantitative assay uses infectious virus of each type to challenge the suspect serum. Cell cultures are then inoculated with the serum-virus mixture and the protective effect of the serum, that is, its ability to neutralize the lethality of the virus, is calculated. The serum neutralization assay is complex and requires a significant amount of technologist time.

Prior to the CDC funding, a pilot study had been begun (late 1999) to determine if West Nile virus has been in Florida in the past. Dr. Arthur Lewis, retired director of the Epidemiology Research Center, returned to the laboratory to assist in the performance of these tests. After preparing seed virus and antiserum for use in the assays, he began a retrospective analysis of past SLE positive human and chicken sera using the neutralization assay. No sera were found positive for antibody to West Nile virus, confirming the etiology as SLE.

Horses seem to be especially sensitive to West Nile virus and have a high case fatality rate. Using the SN assay we were able to show that a horse which had HAI antibody reacting with both SLE and WN in testing at the DACS Kissimmee laboratory, was infected with SLE and not WN.

As part of the WN surveillance project, we are performing viral isolation attempts on dead birds. This is a collaborative effort with the Bureau of Epidemiology, DACS laboratory, County Health Departments, Bureau of Fish & Wildlife and others. People who find dead birds are encouraged to report them on a web site (<http://wld.fwc.state.fl.us/bird/>). Carcasses are sent to the DACS laboratory for necropsy and the tissues are submitted to this laboratory. Since this work began in June, tissues from 56 birds were submitted. They are processed and inoculated into cell culture for virus isolation. Additionally, tissue homogenates are assayed by RT-PCR or NASBA for the direct detection of viral nucleic acid. This is done to provide a rapid preliminary assessment of viral presence, as cell culture requires a long time.

Arbovirus related studies once again comprised a major portion of the 2000 virology workload. Figures 1&2 in the attached powerpoint presentation depict monthly positive sentinels since 1988, for SLE and EEE respectively.

Of the 67 counties in Florida, 27 submitted sera for arbovirus surveillance last season. Degree of participation varied amongst them. Figures 3, 4, 5, 6, and 7 show, respectively, for each county, the numbers of surveillance sites maintained, the total number of susceptible chickens exposed during 2000, the number of serum samples which were submitted from exposed birds, the number of sentinel birds which seroconverted to SLE during 2000, and the number of birds which seroconverted to EEE during 2000. There are still substantial areas of the state which are not monitored. Sampling is clustered, and hence, the distribution of virus activity appears clustered. It would be of value for our understanding and future control of arbovirus outbreaks, if these currently underrepresented areas of the state could be encouraged to continue participation in surveillance activities.

The regional annual seroconversion rates for EEE and for SLE were not significantly above each regional historical mean. Figures 8 and 9 depict the seroconversions to SLE and EEE, respectively, by month, for the four state regions. SLE activity peaked in October and EEE activity peaked in November, but neither was elevated above the historical means during any month. However, seroconversions to EEE occurred sporadically during 8 months of year 2000, including winter months (January, December).

Table 1a provides a listing of the counties which participated during 2000, the numbers of sites and birds they maintained, the number of sera they submitted, the numbers of sentinels which seroconverted to EEE and SLE, the percent of exposed birds which seroconverted. Seroconversion dates are given in table 1b; confirmed seroconversions are indicated by "*"; presumptives are listed when a second (confirming) serum was not provided from that bird.

A total of 41,698 HAI tests were performed for arbovirus studies. This includes both the sentinel flock incidence studies, and wild bird sera submitted for prevalence studies. There were 385 positive tests for SLE and 84 for EEE. Prevalence study samplings were in conjunction with Dr. Jonathon Day (FMEL), and were submitted for identification of major wild amplification and overwintering hosts of these agents in nature. They were funded by a grant from DACS. Additional samples were submitted from Disney Animal Programs and Alabama, Mississippi and Louisiana Health Departments. This aids in our understanding of the natural history of these agents, thus leading to the development of early warning systems and possible outbreak control.

Additionally, sera from human cases of suspect arboviral disease are assayed at our laboratory or at the Jacksonville Laboratory. Clinical sera that have been tested at commercial laboratories are also submitted for confirmation of test result and determination of etiological agent. Submitted

sera are screened by HAI for flavivirus (SLE/WN/DEN) and togavirus (EEE) antibody and are also assayed using a clinical IgM Elisa protocol (SLE/WN/DEN/EEE/CAL). This is done to determine whether antibody detected in the screening assays is due to recent infection, or merely the result of infection or immunization at an undetermined time in the past. If antibody to WNV is detected in a human, a Serum Neutralization Plaque Reduction assay is performed to confirm the presence of antibody specific to WNV. This protocol successfully ruled out WNV as the cause of illness in a Florida resident, after a false positive report from a private laboratory. No WNV antibody positive human cases were detected in Florida in 2000. A number of imported Dengue cases (7 statewide, per Bureau of Epidemiology) were detected in 2000.

It must be noted that this very effective Arbovirus Sentinel Surveillance program is a success because of the efforts of its two full time technical staff, Maribel Casteneda and Rita Schofield. They directed and trained OPS staff working on Arbovirus grants (Bruce Cochrane, Corey Farrell, Robin Stoner) and student volunteers in the HAI protocol, while maintaining test quality control. Our animal care technician, Eddie Tensley, is responsible for the care of our goose flock, and the production of goose erythrocytes used each week as the indicator red blood cell in the HAI assay by both the Tampa and Jacksonville laboratories. The Arbovirus Surveillance Team by working together in such a productive manner has enhanced our ability to provide useful data in a timely manner to a variety of concerned agencies.

Table 1a. ARBOVIRUS SURVEILLANCE REPORT: Sentinel flock activity by county-

County	Number of Sites Monitored	Number of Susceptibles Examined	Number of Sera from Susceptibles Examined	Number of Sentinels Seroconverting		Percent of Sentinels Seroconverting	
				EEE	SLE	EEE	SLE
Alachua	6	36	324	0	0	0.0%	0.0%
Bay	3	28	337	0	0	0.0%	0.0%
Brevard	10	62	708	0	6	0.0%	9.7%
Charlotte	5	38	580	0	9	0.0%	23.7%
Citrus	7	48	412	0	0	0.0%	0.0%
Duval	6	38	324	0	1	0.0%	2.6%
Flagler	4	26	343	1	0	3.8%	0.0%
Hendry	2	31	205	0	3	0.0%	9.7%
Hillsborough	7	57	833	2	6	3.5%	10.5%
Indian River	8	62	1428	0	10	0.0%	16.1%
Lee	17	336	1941	0	71	0.0%	21.1%
Leon	5	40	550	8	0	20.0%	0.0%
Manatee	9	62	1097	0	6	0.0%	9.7%
Martin	5	44	151	0	3	0.0%	6.8%
Okeechobee	3	26	213	0	6	0.0%	23.1%
Orange	20	258	4276	1	0	0.4%	0.0%
Osceola	10	77	544	1	2	1.3%	2.6%
PalmBeach	8	97	804	0	14	0.0%	14.4%
Pasco	3	18	144	0	0	0.0%	0.0%
Pinellas	8	57	674	0	1	0.0%	1.8%
Putnam	6	35	102	2	0	5.7%	0.0%
Sarasota	4	28	381	0	3	0.0%	10.7%
Seminole	4	33	348	0	1	0.0%	3.0%
St. Johns	8	48	235	1	0	2.1%	0.0%
St. Lucie	4	29	347	0	2	0.0%	6.9%
Volusia	4	17	184	0	0	0.0%	0.0%
South Walton	5	44	508	1	0	2.3%	0.0%
Totals	175	1639	17669	17	144		

Table 1b. ARBOVIRUS SURVEILLANCE REPORT: Sentinel flock activity by county

County	Week of Sero Conversion (* Indicates Confirmed)
Alachua	
Bay	
Brevard	10/4(2*), 10/25(2*), 11/6(1*), 11/8(1)
Charlotte	8/11(1*), 8/18(1*), 9/8(1*), 9/15(1*), 9/29(1*), 10/6(1*), 10/20(2*), 10/26(1*)
Citrus	
Duval	10/9(1*)
Flagler	5/26(1*)
Hendry	8/21(1*), 9/5(1*), 9/18(1),
Hillsborough	EEE: 2/28(1*), 12/18(1*); SLE: 9/25(1*), 10/9(1*), 10/16(1*), 11/13(1*), 11/27(1*), 12/4(1*)
Indian River	9/28(1*), 10/5(2*), 10/12(1*), 10/19(5*), 11/2(1*)
Lee	7/11(1*), 8/7(3*), 8/21(3*), 8/29(2*), 9/4(3*), 9/5(2*), 9/11(1*,1), 9/18(2*,2), 9/19(4*), 9/25(7*,1), 10/2(6*), 10/3(1*), 10/10(3*), 10/16(7*,2), 10/17(8*), 10/23(2*), 10/30(3*,2), 10/31(3*), 11/3(1*n), 11/6(1*)
Leon	10/16(1*), 11/6(1*), 11/13(5*), 11/20(1*)
Manatee	8/25(1*), 9/8(1*), 10/19(1*), 11/17(1*), 12/1(1*), 12/15(1*)
Martin	1/9(1*), 10/16(1*), 11/6(1*)
Okeechobee	10/8(2*), 10/9(1*), 11/20(3*)
Orange	1/20(1*)
Osceola	EEE: 2/24(1*); SLE: 1/6(1*), 9/26(1*)
PalmBeach	9/25(1*), 10/9(4*), 10/16(1*), 10/23(2*), 10/30(2*), 11/13(1*, 3*n)
Pasco	
Pinellas	11/20(1)
Putnam	8/7(1*), 8/12(1*)
Sarasota	9/25(2*), 10/2(1*)
Seminole	11/3(1*)
St. Johns	9/22(1*)
St. Lucie	10/26(2*)
Volusia	
South Walton	10/9(1*)