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**Arbovirus Surveillance:
Annual Summary Report
2001**

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Annual Arbovirus Surveillance Summary Report: 2001

This past arbovirus season was extraordinary. Because of the emergence of West Nile virus (WNV), an arbovirus new to the Americas, in the northeast in 1999 and its apparent migration down the east coast, surveillance efforts had been intensified through funding by a supplemental ELC grant from the CDC. Our longstanding laboratory experience with arboviruses and our state multi-agency collaborative surveillance program placed Florida in a better position to face the onslaught of West Nile Virus than were many states, however, it was a challenge to meet the testing demand that was so much greater than anticipated. Surveillance activity described below includes assay of dead bird and mammal tissue (virus detection and isolation) and sentinel chicken and wild bird serology assays performed at the Florida Department of Health, Bureau of Laboratories, Tampa Virology Laboratory. Results of clinical testing are not presented in this report.

West Nile virus (WN) is a flavivirus, closely related to SLE but unlike SLE, birds infected with WNV often die. Virus may be detected in the tissues of those birds. We detected the first infected bird in Florida on July 3, 2001, in a crow submitted from Jefferson County. Between January 1, 2001 and July 6, the date of the first announcement of WNV detection, 113 dead birds had been received for analysis. During the next 6 months, 7,773 specimens were received for analysis. This is illustrated in figure 1.

Initially, birds from throughout the State were sent to the Florida Department of Agriculture and Consumer Services (DACS) Animal Disease Laboratory in Kissimmee for necropsy, and the tissues then sent to our laboratory for assay, leading to significant delays in testing. In September, DACS assigned a crew of technicians to our laboratory for temporary duty and necropsy space was allocated. In October, DoH necropsy technician positions (OPS) were funded through the ELC supplemental grant and two were hired. They were trained by the DACS technicians and in November the DACS technicians were released back to their unit. Having these necropsy technicians on staff has improved turn-around-time and our ability to process samples efficiently. Nevertheless, without the participation of DACS at the inception of this outbreak, it would not have been possible to process and examine the large number of samples received.

All of the submitted animals were screened for WNV by molecular assays (TaqMan RT-PCR) by the end of 2001. Samples negative by screening were inoculated onto VERO cell culture for detection of other arboviruses and to increase detection sensitivity for WNV. Molecular methods are highly sensitive, but only assay a very small amount of sample; cell culture is also a very sensitive technique and allows for the testing of a much larger sample volume. However, cell culture is very slow and about 2000 samples remain to be completed by this assay. This effort will continue during the early part of 2002.

A total of 7,773 specimens (7,675 birds, 98 mammals) were received for virological assay during 2001. Submissions were made from all counties in Florida. To date, WNV has been detected in 1106 birds and 7 mammals; EEE in 17 birds and 5 mammals. This number will increase somewhat as we complete testing of samples in cell culture. Data on submissions and results by county is presented in Table 1. Positive bird data was faxed to the counties as expeditiously as possible. As the number of sample submissions was more than triple our original estimates, reporting was hampered as we struggled to develop a computer database and

reporting system as well as a Quality improvement plan to prevent reporting errors. Work began on developing an "Access" database to process year 2002 data.

Last year 1,378 pools of mosquitoes were submitted from 13 counties across the state. Molecular (TaqMan) assays and/or cell culture inoculation have been performed on 1373 of them. It is important to determine whether the virus detected in a mosquito is viable (i.e., multiplies in cell culture) as part of ascertaining vector potential of the various species. WNV was detected in 10 pools: 1 each from Bay, Madison and Washington counties, 2 from Pinellas and 4 from Monroe county. Four WNV isolates were made in cell culture (*Cx. nigripalpus*, *D. cancer*, *Oc. atlanticus*, *Oc. taeniorhynchus*); 6 were positive by molecular screening (*An. atropos*, *An. crucians*, *Cs. melanura*, *D. cancer*, *Oc. taeniorhynchus* (2)). One pool (*Cx. quinquefasciatus*) yielded a EEE isolate; 16 as yet unidentified viruses (probably California group) were also isolated in VERO cell culture.

The "Sentinel Chicken" surveillance program has long been a mainstay of arbovirus surveillance in Florida. It has proven its usefulness in predicting risk for St. Louis Virus infection. This was its the first year for detection of antibody to West Nile Virus. Mosquito control agencies or County 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 Flavivirus (SLE/WNV) and to Eastern Equine Encephalitis (EEE) virus. Reports were expeditiously faxed to the submitters each Friday afternoon. Summary reports were also compiled and e-mailed to the County Health Department Directors and other interested parties each week.

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. An additional advantage of the HAI assay, is that serum from any species can be analyzed by this method. Thus, our surveillance program is capable of detecting the presence of both of these arboviruses in sentinel chickens, wild birds and mammals. 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; some cross-reactions may occur in this assay. Sentinel sera which reacted with SLE antigen in the HAI test were deemed "flavivirus positive" and if sufficient residual serum was available, it was assayed by the "Chicken Elisa" protocol as developed by CDC. Confirmation of antibody to WNV in other species requires the serum neutralization assay.

The most specific test to distinguish between antibodies developed to these viruses is the serum neutralization 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, it's ability to neutralize the lethality of the virus, is calculated. The serum neutralization assay is complex, hazardous and requires a significant amount of technologist time.

Horses seem to be especially sensitive to West Nile virus and have a high case fatality rate. Horse brains submitted for rabies examination were also tested for the presence of WNV by molecular assays. Sera from 179 mammals, mostly horses, were submitted for arbovirus antibody determination (HAI, SNPR assays). Sera from wild-trapped birds (1257 birds, 12

positive for antibody to flavivirus, 89 positive for antibody to EEE) were also included in serological (HAI, SN) assays for the detection of antibody to WNV.

Figures 2 & 3 depict numbers of monthly positive sentinel seroconversions since 1988, for EEE and Flavivirus (SLE/WNV) respectively.

Of the 67 counties in Florida, 31 submitted sera for arbovirus surveillance last season. Degree of participation varied amongst them. Figures 4, 5, 6, 7, 8, and 9 show, respectively, for each county: the numbers of surveillance sites maintained, the total number of susceptible chickens exposed during 2001, the number of serum samples submitted from exposed birds, the number of sentinel birds that seroconverted to EEE, the number of birds that seroconverted to SLE and the number of birds that seroconverted to WNV during 2001. 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.

Table 2a provides a listing of the counties which participated during 2001, the numbers of sites and birds they maintained, the number of sera they submitted, the numbers of sentinels which seroconverted to EEE, SLE and WNV and the percent of exposed birds which seroconverted. Seroconversion dates are given in table 2b; confirmed seroconversions are indicated by "*"; presumptives are listed when a second (confirming) serum was not provided from that bird.

The annual regional seroconversion rates for EEE and SLE were below each regional historical mean. This is the first year WNV has been detected in Florida, thus no historical comparisons are possible. Figures 10, 11 and 12 depict the seroconversions to EEE, SLE and WNV, respectively, by month, for the four state regions. Flavivirus activity, comprised of antibody response to WNV, peaked in October, as was normally seen for SLE. Total flavivirus activity was below average for the south and central regions, but significantly elevated in the north and panhandle regions of the state. Seroconversions to EEE occurred during 9 months of year, rising above the statewide monthly average only in May.

A total of 56,458 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 549 positive tests for flavivirus and 336 for EEE.

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 (Corey Farrell, Robin Stoner, Sanjeev Thomas, Bruce Cochrane) and student volunteers in the HAI protocol, while maintaining test quality control. Eddie Tensley, is responsible for 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, and assists in specimen preparation. 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 1. Dead bird/mammal submissions for detection of WNV, by county

county	A	B	C	D	county	A	B	C	D
Alachua	294	3.8	66	5.9	Lee	18	0.2	2	0.2
Baker	5	0.1	2	0.2	Leon	318	4.1	110	9.9
Bay	446	5.7	85	7.6	Levy	94	1.2	23	2.1
Bradford	63	0.8	19	1.7	Liberty	17	0.2	9	0.8
Brevard	307	3.9	3	0.3	Madison	35	0.5	10	0.9
Broward	185	2.4	14	1.3	Manatee	88	1.1	4	0.4
Calhoun	16	0.2	8	0.7	Marion	188	2.4	45	4.0
Charlotte	69	0.9	8	0.7	Martin	37	0.5	3	0.3
Citrus	97	1.2	15	1.3	Monroe	165	2.1	18	1.6
Clay	243	3.1	51	4.6	Nassau	98	1.3	24	2.2
Collier	128	1.6	7	0.6	Okaloosa	410	5.3	25	2.2
Columbia	51	0.7	29	2.6	Okeechobee	19	0.2	1	0.1
Dade	397	5.1	21	1.9	Orange	262	3.4	3	0.3
Desoto	7	0.1	0	0.0	Osceola	46	0.6	2	0.2
Dixie	35	0.5	9	0.8	Palm Beach	189	2.4	7	0.6
Duval	537	6.9	103	9.3	Pasco	249	3.2	21	1.9
Escambia	216	2.8	9	0.8	Pinellas	207	2.7	1	0.1
Flagler	74	1.0	2	0.2	Polk	61	0.8	6	0.5
Franklin	22	0.3	4	0.4	Putnam	92	1.2	12	1.1
Gadsen	53	0.7	27	2.4	Santa Rosa	162	2.1	16	1.4
Gilchrist	38	0.5	11	1.0	Sarasota	166	2.1	4	0.4
Glades	19	0.2	1	0.1	Seminole	203	2.6	4	0.4
Gulf	41	0.5	15	1.3	St. Johns	33	0.4	0	0.0
Hamilton	31	0.4	14	1.3	St. Lucie	37	0.5	2	0.2
Hardee	2	0.0	0	0.0	Sumter	35	0.5	1	0.1
Hendry	13	0.2	2	0.2	Suwanee	102	1.3	47	4.2
Hernando	129	1.7	8	0.7	Taylor	29	0.4	13	1.2
Highlands	49	0.6	2	0.2	Union	32	0.4	8	0.7
Hillsborough	157	2.0	2	0.2	Volusia	84	1.1	3	0.3
Holmes	36	0.5	13	1.2	Wakulla	108	1.4	64	5.8
Indian River	12	0.2	0	0.0	Walton	84	1.1	8	0.7
Jackson	98	1.3	12	1.1	Washington	41	0.5	16	1.4
Jefferson	27	0.3	16	1.4	unknown	6	0.1	1	0.1
Lafayette	15	0.2	8	0.7	total	7773	100.0	1113	100.0
Lake	146	1.9	14	1.3					

Column Headings:

A = number of specimens (birds & mammals) submitted by the county for virus detection

B = % of statewide total number of samples (7773) submitted by the county

C = number of county samples positive for West Nile Virus

D = % of statewide total of WNV positive samples (1113) submitted by the county

Table 2a. ARBOVIRUS SURVEILLANCE REPORT: Sentinel flock activity by county-

County	# of Sites monitored Monitored	# of Susceptibles Examined Examined	# of Sera from Susceptibles Examined	Number of Sentinels Seroconverting			Percent of Sentinels Seroconverting		
				EEE	SLE	WN	EEE	SLE	WN
Alachua	7	71	552	7	0	20	9.9%	0.0%	28.2%
Bay	3	35	472	4	0	15	11.4%	0.0%	42.9%
Brevard	10	67	686	0	1	1	0.0%	0.1%	1.5%
Charlotte	5	34	809	0	0	0	0.0%	0.0%	0.0%
Citrus	7	67	492	0	0	3	0.0%	0.0%	4.5%
Collier	5	46	297	0	0	6	0.0%	0.0%	13.0%
Desoto	1	6	54	0	0	0	0.0%	0.0%	0.0%
Duval	7	59	395	4	0	34	6.8%	0.0%	57.6%
Flagler	4	60	298	6	2	0	10.0%	0.7%	0.0%
Hendry	2	24	212	0	0	0	0.0%	0.0%	0.0%
Hillsborough	8	107	967	2	0	2	1.9%	0.0%	1.9%
Indian River	8	53	1672	0	0	3	0.0%	0.0%	5.7%
Lee	17	256	1575	0	1	4	0.0%	0.1%	1.6%
Leon	8	99	896	12	0	54	11.1%	0.0%	54.5%
Manatee	8	101	954	0	0	0	0.0%	0.0%	0.0%
Martin	5	40	310	0	0	8	0.0%	0.0%	20.0%
Okeechobee	3	21	215	0	0	2	0.0%	0.0%	9.5%
Orange	19	249	4731	13	0	3	5.2%	0.0%	1.2%
Osceola	9	90	1003	1	0	2	1.1%	0.0%	2.2%
PalmBeach	6	77	1156	0	2	3	0.0%	0.2%	3.9%
Pasco	3	21	317	0	0	1	0.0%	0.0%	4.8%
Pinellas	8	66	1012	0	0	2	0.0%	0.0%	3.0%
Polk	8	117	256	0	1	1	0.0%	0.4%	0.9%
Putnam	6	40	266	2	0	8	5.0%	0.0%	20.0%
Sarasota	6	36	855	0	0	0	0.0%	0.0%	0.0%
Seminole	5	54	627	1	0	1	1.9%	0.0%	1.9%
St. Johns	9	100	481	2	0	20	2.0%	0.0%	20.0%
St. Lucie	5	38	618	0	0	0	0.0%	0.0%	0.0%
Suwanee	1	16	49	0	0	3	0.0%	0.0%	18.8%
Volusia	6	27	566	0	0	0	0.0%	0.0%	0.0%
South Walton	5	51	772	11	0	6	21.6%	0.0%	11.8%
Totals	204	2128	23565	65	7	202			

Table 2b. ARBOVIRUS SURVEILLANCE REPORT: Sentinel flock activity by county

County	Week of Sero Conversion (* Indicates Confirmed)
Alachua	EE: 7/16(1*), 7/24(1*), 7/30(1*), 9/4(1*), 10/29(3); WN: 9/24(8*), 10/9(1*), 10/15(6*), 10/22(4*), 10/29(1*)
Bay	EE: 8/14(2*), 8/21(1*), 9/11(1*); WN: 8/21(2*), 9/18(3*), 9/25(2*), 10/23(2*), 11/02(1*), 11/13(1*), 11/27(1*), 12/4(1*), 12/18(2*)
Brevard	SLE: 7/12(1*); WN: 11/02(1*)
Charlotte	
Citrus	WN: 10/8(1*), 11/05(1*), 11/13(1*)
Collier	WN: 10/29(1*), 11/14(1*), 11/19(1*),12/4(1*,1n), 12/18(1n)
Desoto	
Duval	EE: 7/24(2*), 8/7(1*), 8/20(1*); WN: 7/10(1*), 8/20(1*), 9/4(1*), 9/10(4*), 9/14(1*,1n), 9/17(9*), 9/24(7*), 10/1(5*,1n), 10/15(2*), 10/29(1*)
Flagler	EE: 5/11(1*), 5/24(1*), 6/22(1*), 6/28(1), 7/12(1n), 8/23(1*); SLE: 5/31(1), 6/15(1);
Hendry	
Hillsborough	EE: 4/23(1*), 11/05(1*); WN: 10/29(1*), 11/05(1*)
Indian River	WN: 11/08(1*), 11/15(1*), 11/21(1*)
Lee	SLE: 2/26(1*); WN: 11/06(3*), 12/11(1*)
Leon	EE: 8/7(2*), 8/13(1*), 8/27(1*), 9/10(1*), 10/1(1), 10/15(3*), 10/29(1*),11/05(1*,1); WN: 7/24(1*), 8/13(1*), 8/20(1*), 8/27(3*), 9/4(1*), 9/10(3*,1), 9/18(8*), 9/24(11*), 10/2(1*), 10/8(12*), 10/15(2*), 10/22(3*), 10/29(2*,1), 11/05(1*), 12/10(1), 12/17(1)
Manatee	
Martin	WN: 9/17(2*), 9/24(1*), 10/8(1*), 10/15(1*), 10/22(1*), 10/29(2*)
Okeechobee	WN: 11/05(1*), 11/19(1*)
Orange	EE: 5/29(5*), 5/31(2*), 6/19(1*), 11/29(1*), 12/11(1*), 12/14(1*), 12/26(2); WN: 11/29(2*), 12/6(1*)
Osceola	EE: 11/13(1*); WN: 10/8(1*), 10/15(1*)
PalmBeach	SLE: 9/17(1*), 10/08(1*); WN: 10/8(2*), 12/17(1*)
Pasco	WN: 10/9(1*)
Pinellas	WN: 10/22(1*), 11/05(1*)
Polk	SLE: 8/24(1n); WN: 9/7(1*)
Putnam	EE: 7/30(1*), 8/6(1*); WN: 8/21(1*), 9/17(2*), 9/24(4*), 9/28(1*)
Sarasota	
Seminole	EE: 12/7(1*); WN: 10/26(1*)
St. Johns	EE: 7/12(1*), 11/01(1*); WN: 9/20(3*), 10/4(4*), 10/18(5*), 10/25(1*), 11/08(4*), 11/21(1*), 11/29(1*), 12/10(1*)
St. Lucie	
Suwanee	WN: 10/11(2*), 10/24(1*)
Volusia	
South Walton	EE: 7/30(2*), 8/20(1*), 8/27(2*), 9/10(1*), 11/05(1*), 11/13(2*), 11/19(1*), 12/03(1*); WN: 10/8(2*), 10/15(2*), 10/29(1*), 12/3(1)

Figure 1: Tissues specimens submitted for detection of West Nile Virus, by week.

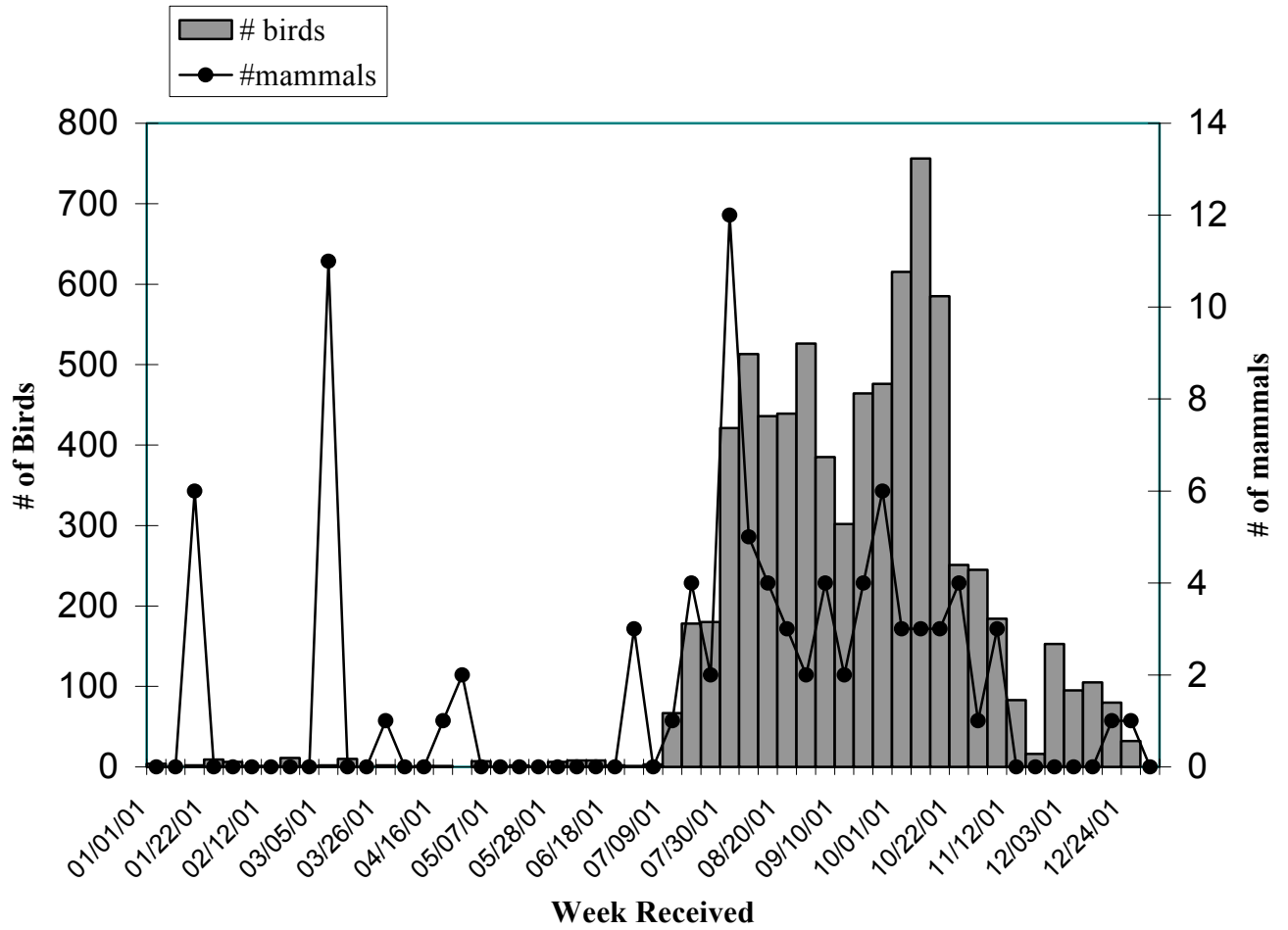


Figure 2.

EEE Seroconversions by Month from 1988-2001

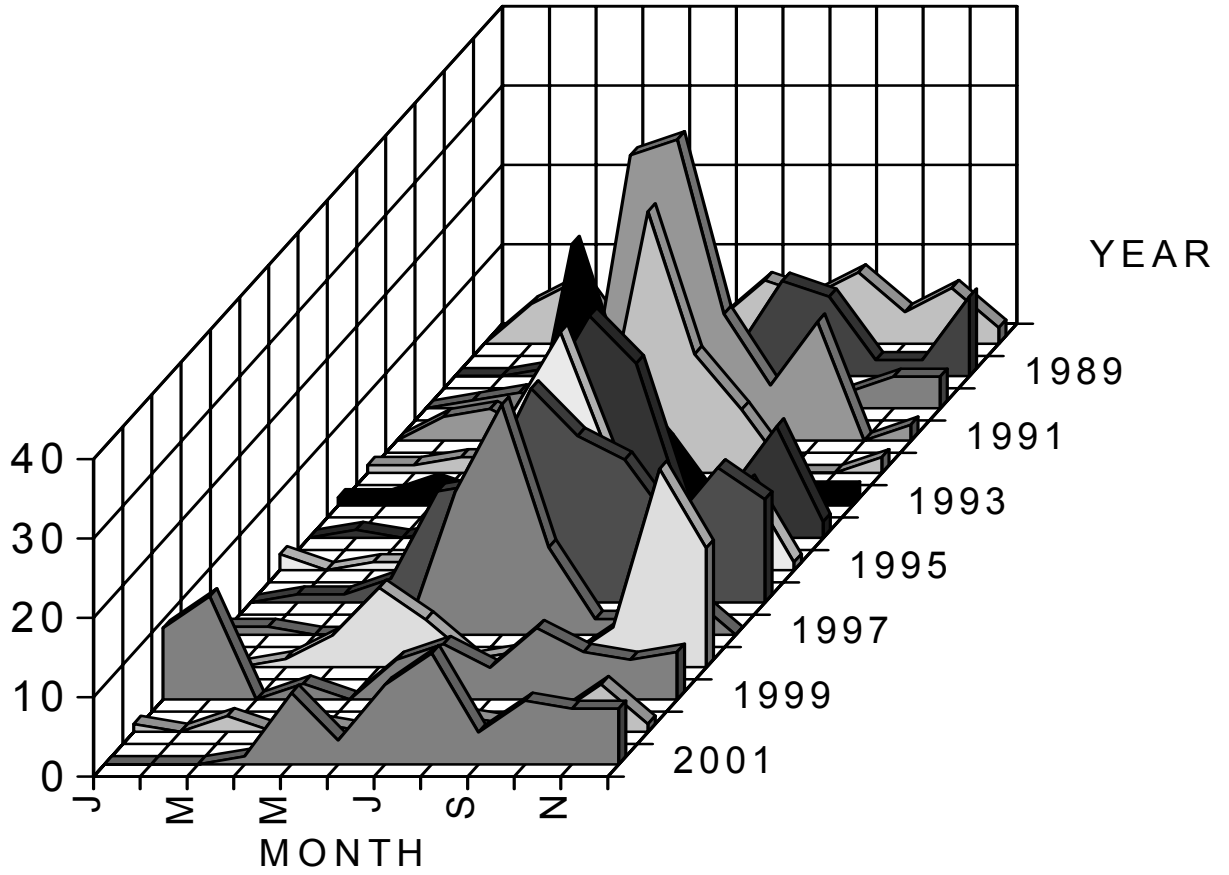


Figure 3.

Flavivirus (SLE/WN) Seroconversions by Month from 1988-2001

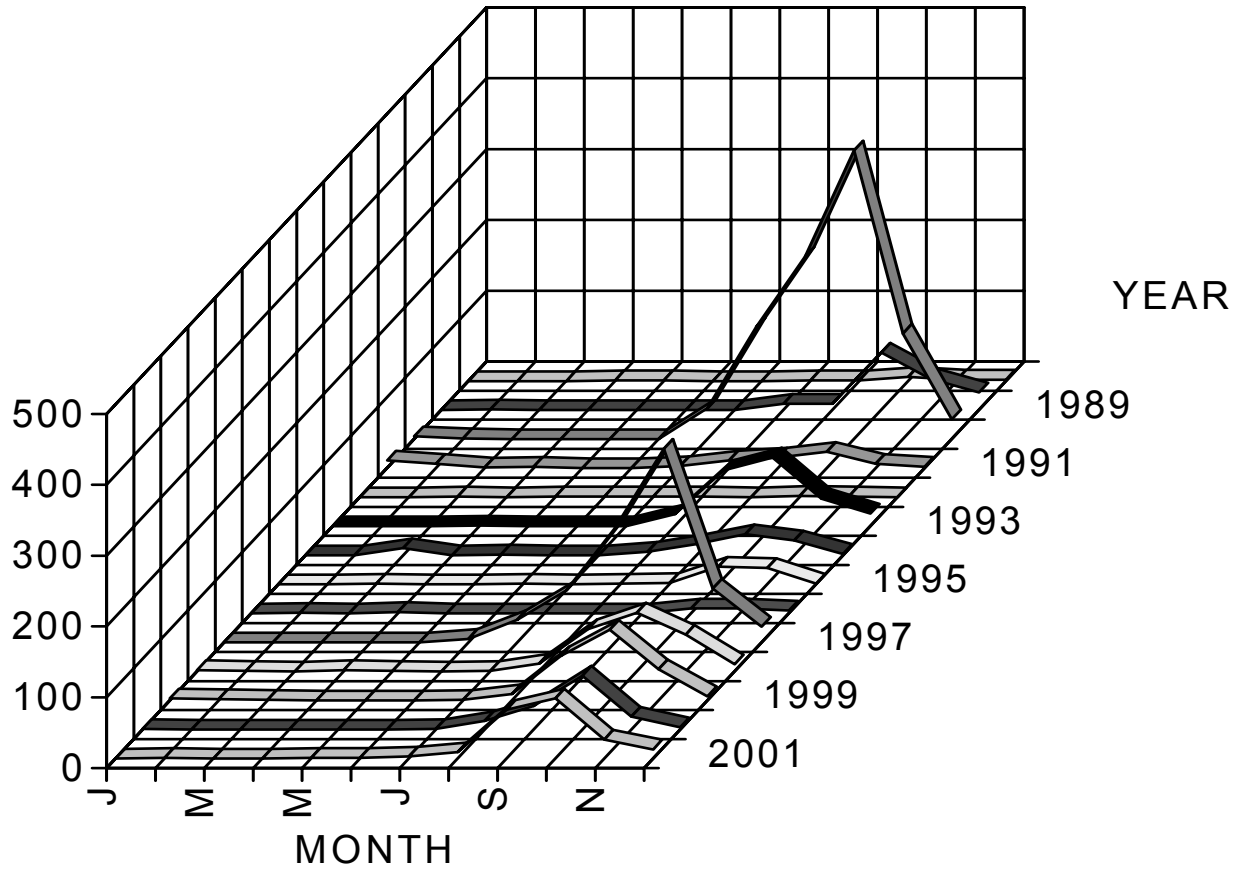


Figure 4.

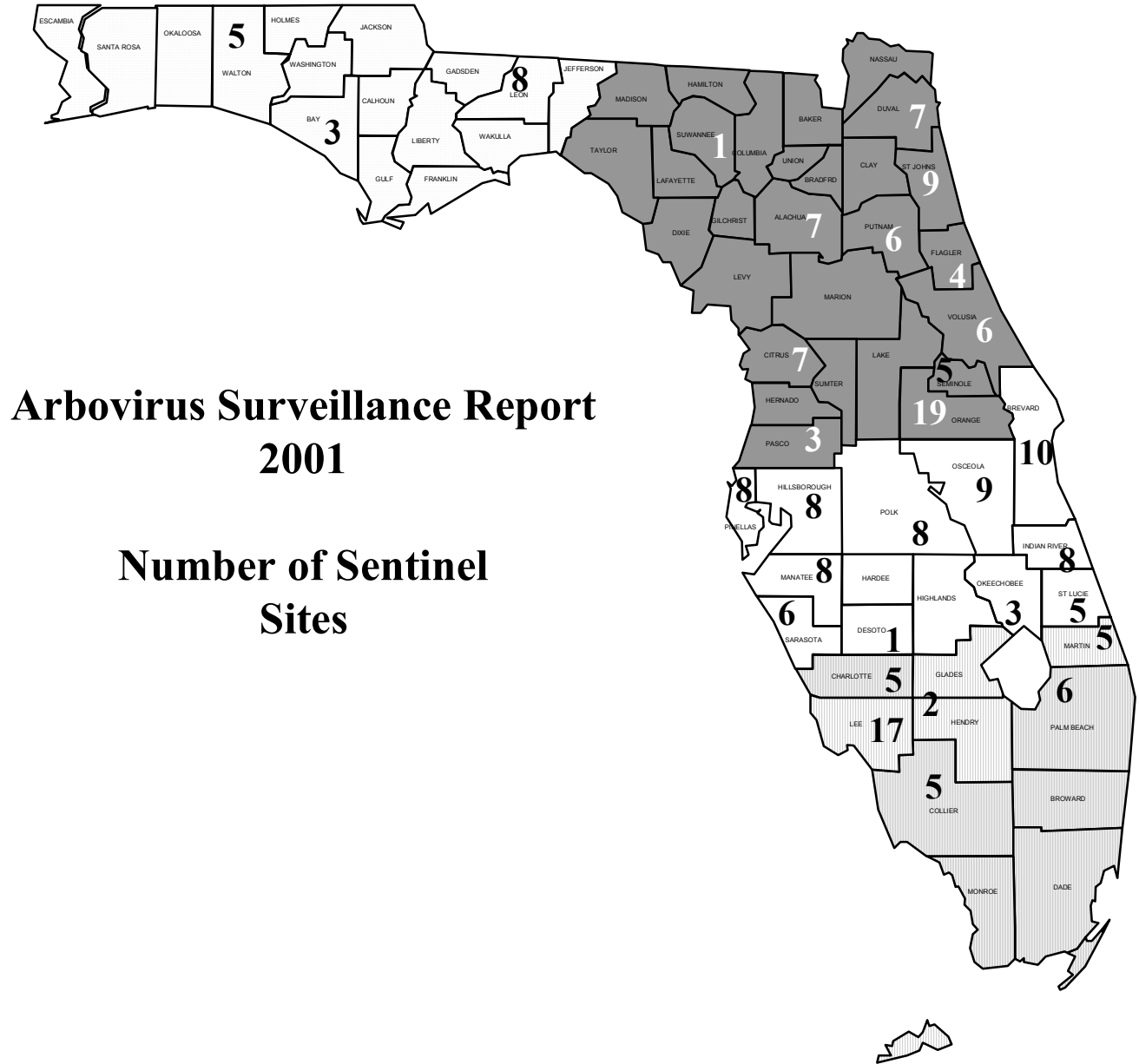
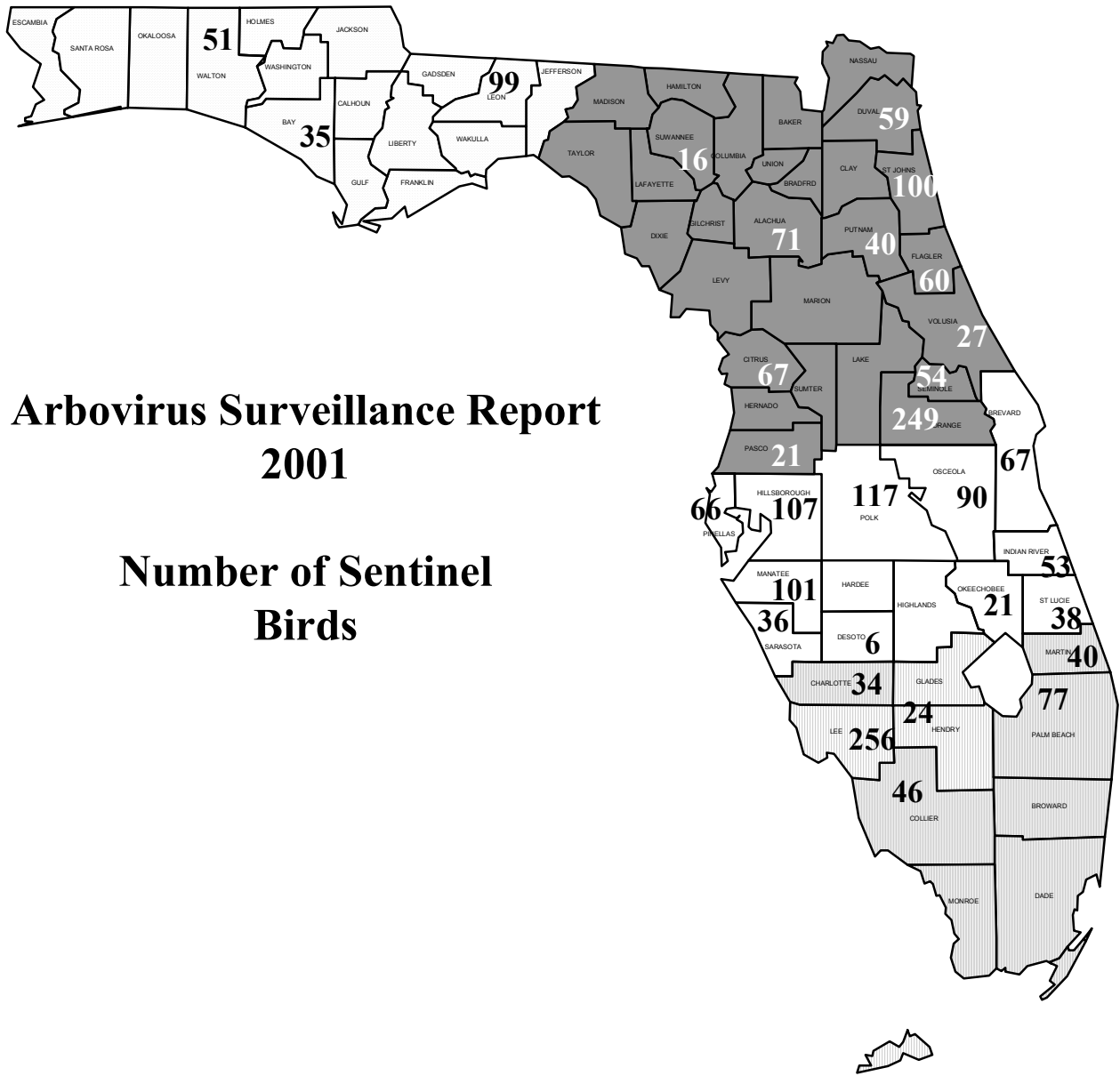


Figure 5



**Arbovirus Surveillance Report
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**Number of Sentinel
Birds**

Figure 7

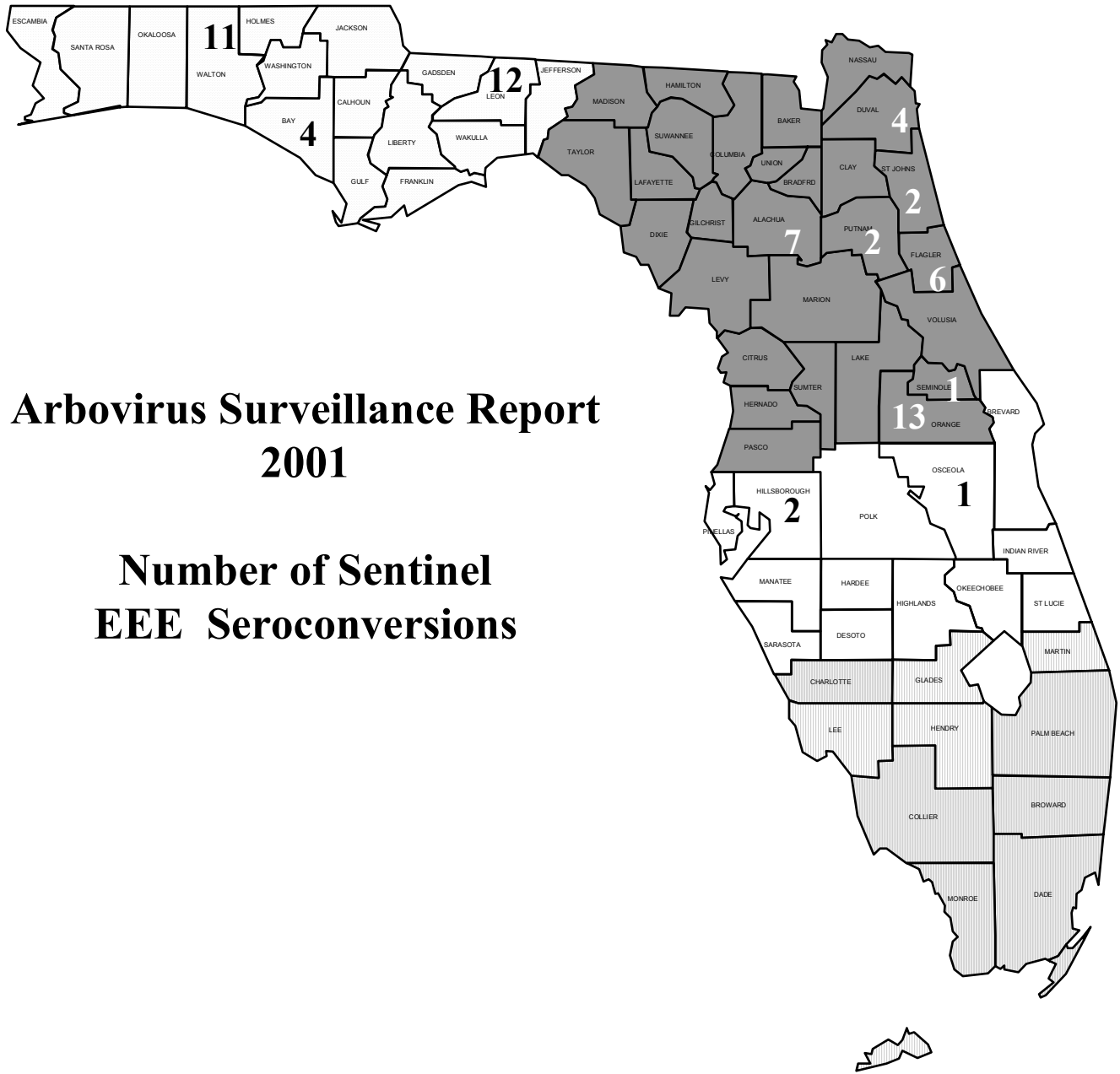


Figure 8.

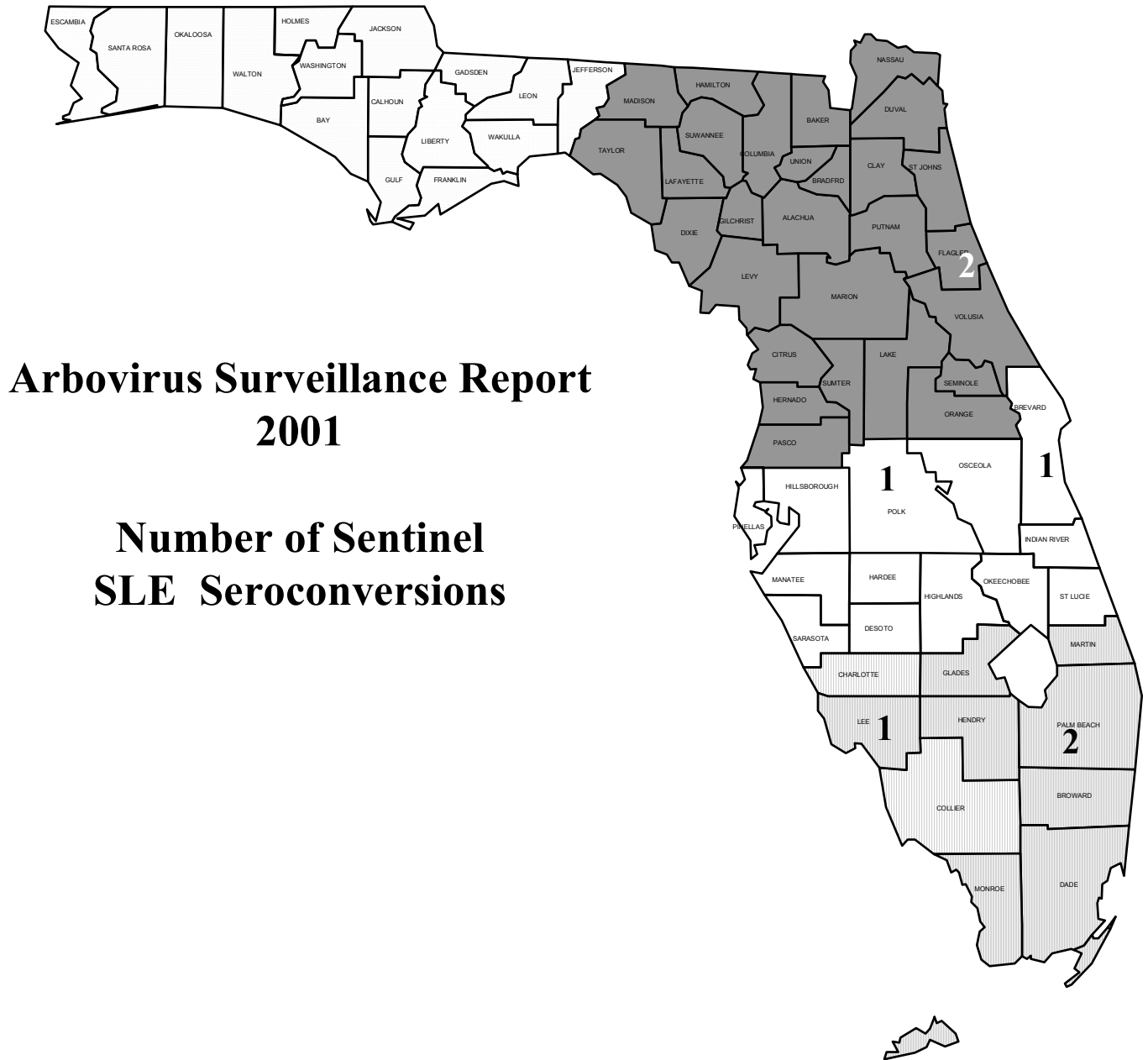


Figure 9.

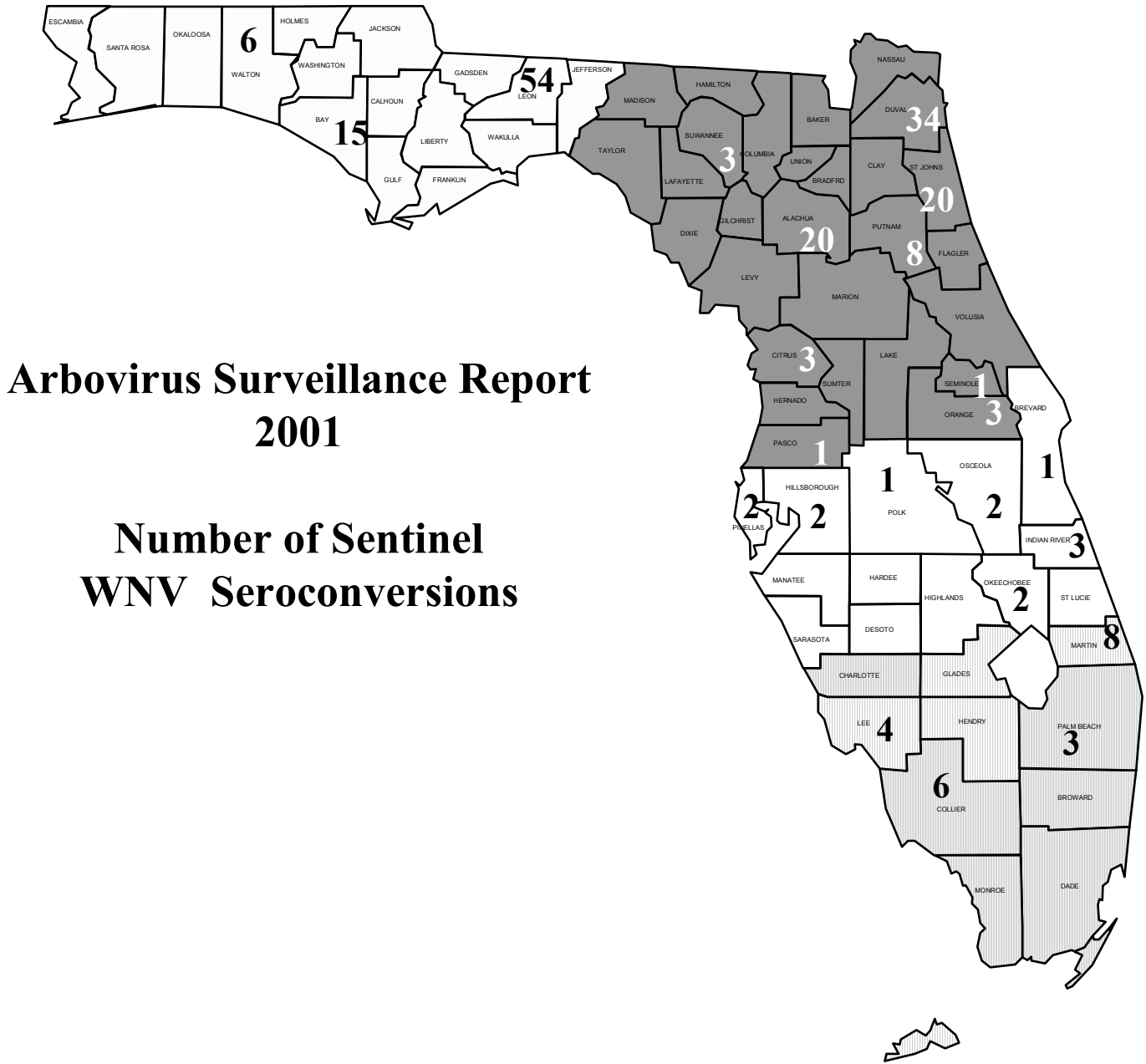


Figure 10.

Arbovirus Surveillance Report: Florida 2001 Number of Sentinel Seroconversions for EEE by Month

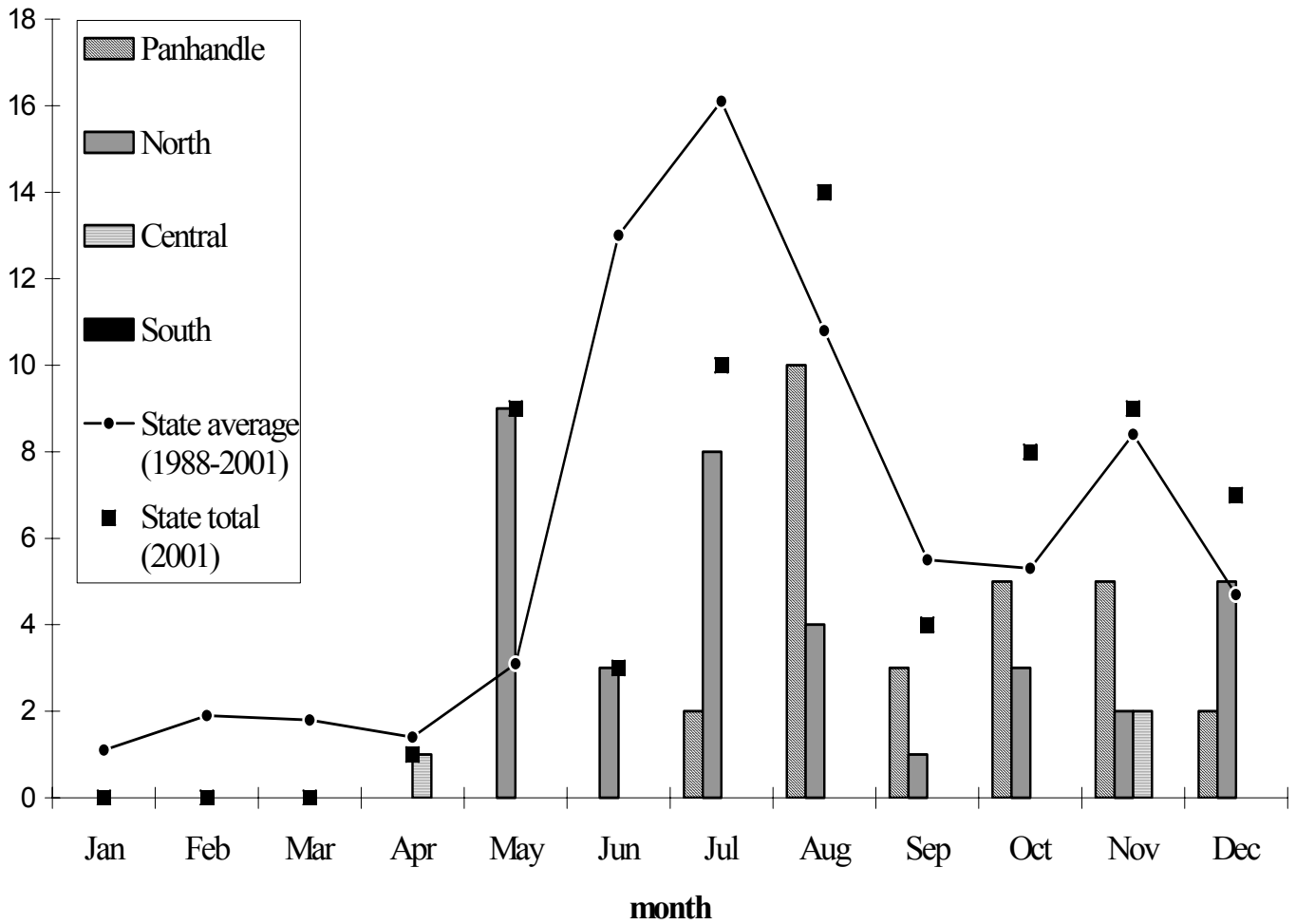


Figure 11.

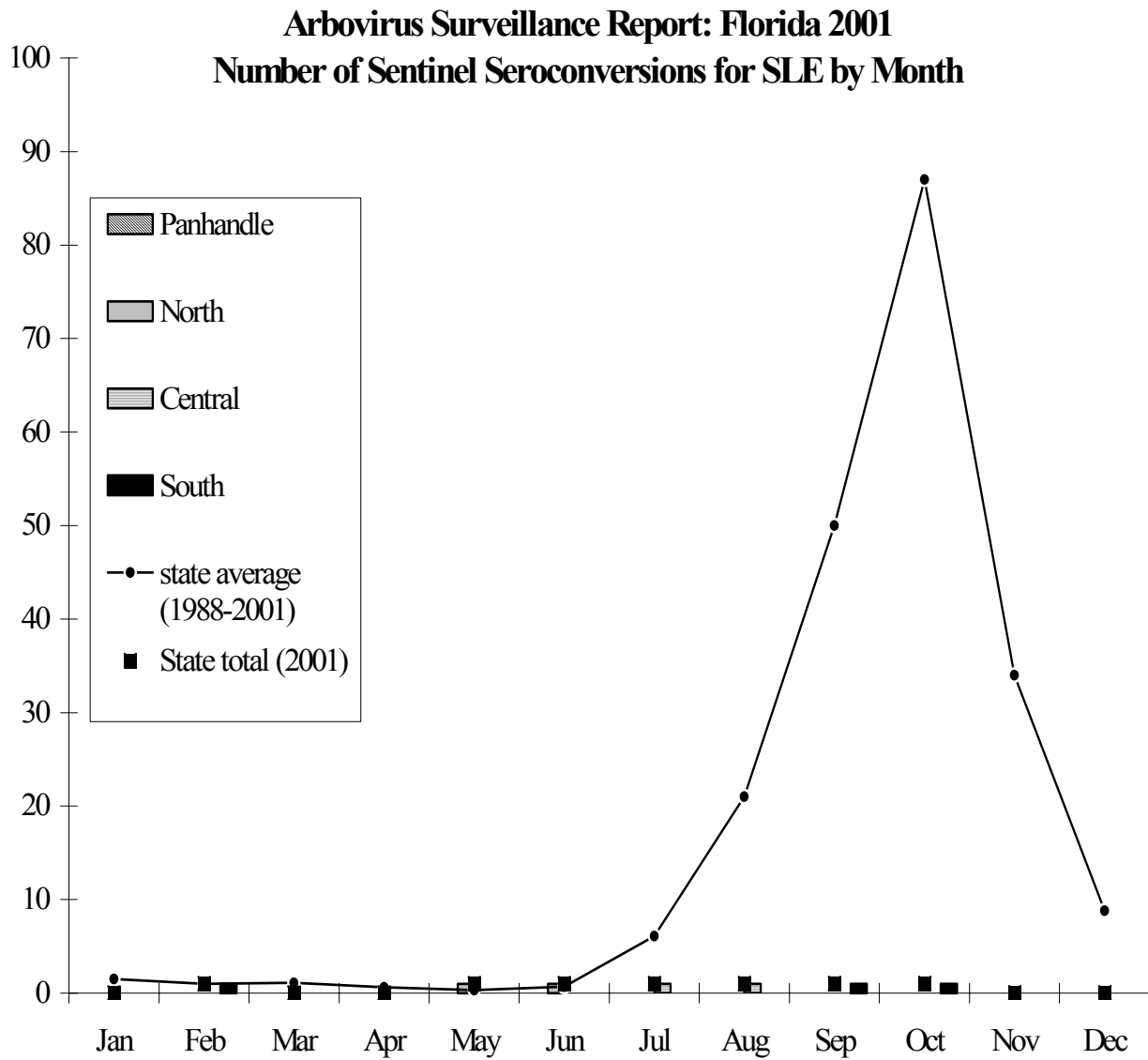


Figure 12.

