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Arbovirus Surveillance: Annual Summary Report 2003

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Annual Arbovirus Surveillance Summary Report: 2003

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 rapid migration, surveillance efforts had been intensified with funding through a supplemental ELC grant from the CDC. Our longstanding laboratory experience with arboviruses and multi-agency collaborative surveillance program placed Florida in a better position to face the onslaught of West Nile Virus then were many states. Surveillance 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 (human diagnostic) testing are not included 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. The first infected bird in Florida was detected on July 3, 2001, in a crow submitted from Jefferson County. At this time the virus is present throughout Florida, although activity in some parts of the state is higher than in others. Submissions of animal tissue specimens for virus detection, by week, is illustrated in figure 1. Most samples were received August through October.

All of the submitted animals were screened for WNV by molecular assays (TaqMan RT-PCR), usually within 1 week of receipt. 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. Cell culture assays, which require a minimum of 2 weeks, will be completed for all samples by mid January 2004.

A total of 2,401 specimens (2,320 birds, 78 mammals, 3 reptiles), were received for virological assay during 2003. This is a decrease of 42% in dead animal submissions from 2002. Submissions were made from most counties in Florida. Mammals included: 59 horses, 4 deer, 4 panthers, 2 baboons, 2 squirrels, and 1 each bat, cow, ferret, fox, goat, rabbit and raccoon. To date, WNV has been detected in 486 birds and 2 mammals (horses); EEE in 19 birds and 28 mammals (horses). Highlands J virus was isolated from 2 birds and a picorna-like virus was isolated from 2 baboons. Data on submissions and WN test results for 2003, 2002 and 2001 by county is presented in Table 1.

During 2003, 6,292 pools of mosquitoes were submitted from 20 counties across the state. Molecular (TaqMan) assays for WNV were performed on all submitted pools. It is desirable 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. Additionally, extracts of some mosquitoes seem to contain inhibitors of RT-PCR, resulting in false negative molecular assays. Thus VERO cell cultures were also inoculated with homogenized pools.

A commercial test for WNV antigen in mosquito pools (VecTest) was used by some mosquito control agencies. They sent residual samples from 427 VecTest one step kits to TBL for confirmation. Because these samples are inactivated, they can be tested only by RT-PCR; cell

culture is not possible. A two-step VecTest is available which allows for both cell culture and RT-PCR confirmation; 164 specimens from two-step kits were submitted.

WNV was detected in 42 traditional pools: Escambia (1), Monroe (10), Orange (1) and Palm Beach (30) counties; 1 pool from Escambia county was positive for EEE. WNV was detected in 2 VecTest two-step samples from Escambia County. 3 WNV isolates were made only in cell culture (*Cx. nigripalpus, Cx. quinquefasciatus, Oc. taeniorhynchus*); 21 were positive only by molecular screening (*Cx. nigrapalpus, , Cx. quinquefasciatus, Oc. taeniorhynchus*) and 18 were positive by both methods (*Cx. erraticus, Cx, nigrapalpus, Cx. quinquefasciatus, Oc. taeniorhynchus*). One pools (*Oc. infirmatus*) yielded an EEE isolate; 3 as yet unidentified viruses (probably California group) were also isolated in VERO cell culture.

Collier County submitted 142 pools that had been tested by VecTest during August. They had determined that 4 pools were WN positive, and 11 pools were equivocal positive, with faint bands for both SLE and WN. Molecular assays (TaqMan RT-PCR) for WN virus were performed in our laboratory on all pools and equivocal pools were also tested for SLE. No pools were SLE positive; 1 VecTest WN positive pool was negative by RT-PCR. WN virus was detected in 7 of the VecTest negative pools. Of the equivocal pools, 3 were WN positive by PCR, the remaining 8, were negative. Thus, in this study, if the equivocal test results are considered as positive, VecTest sensitivity was 46%, specificity 93%, predictive value of a positive test (PVP) 40% and predictive value of a negative test (PVN) 94%. If the VecTest equivocals are considered negative, the results are 23%, 99%, 75% and 93% respectively.

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 second 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 for all participating counties. The Department of Health used this data in its determination of whether to issue/call off Medical Alerts.

The HAI test used in this laboratory is a broadly reactive screening test, and detects total antibodies developed to WN, as well as to SLE and EEE. 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, mammals and reptiles. Nevertheless, to ascertain which virus is present requires additional testing.

The IgM Elisa assay provides a means to distinguish between antibody to SLE or to WN 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, were assayed by the Chicken IgM Elisa the week following the HAI test, with results reported on Wednesday. Since IgM to WN in chickens

is of short duration (few weeks), ELISA negative or equivocal sera were then re-tested using a serum neutralization assay. 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, its 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.

Of the 4,361 individual sentinel birds assayed this year (44,434serum samples), 1,346 (30.3%) developed antibody to WN, 313 (7.2%) to EEE and 11 (0.02%) to SLE virus. Sera from 74 mammals were submitted for arbovirus antibody determination (HAI, SNPR assays). Sera from 1272 non-sentinel avians were also assayed in (HAI, SN) assays for the detection of antibody to WNV. Data are presented in table 2. Of these birds, 5 had antibody to both WN and EEE. Sera from 204 reptiles, both farmed and wild-trapped alligators and crocodilians, were assayed for antibody to WN. Antibody was detected in 9 of 33 wild and 10 of 20 farmed alligators submitted.

Figures 2a and 3a depict numbers of monthly positive sentinel seroconversions since 1988, for EEE and flavivirus (SLE/WNV) respectively. Figures 2b and 3b depict rates of seroconversion. As the numbers of sentinels has changed significantly over the years, for a meaningful interpretation of the data, it is essential that rates of seroconversion rather than just numbers of positive birds be compared.

Of the 67 counties in Florida, 35 submitted sera for arbovirus surveillance last season. This is an increase from 27 counties participating in 2001. Degree of participation varied amongst the regions. Figure 4a depicts the # of sentinels exposed in each region of the state by month, 4 b the number of sentinel sera submitted. Figures 5, 6, 7, 8, 9 and 10 show, respectively, for each county: the numbers of surveillance sites maintained, the total number of susceptible chickens exposed during 2003, the number of serum samples which were submitted from exposed birds, the number of sentinel birds which seroconverted to EEE, the number of birds which seroconverted to SLE and the number of birds which seroconverted to WNV during 2003.

There are still substantial areas of the state that are not monitored. Sampling is clustered, and hence, the distribution of virus activity appears clustered. The panhandle and southern regions had the lowest level of sentinel participation and, in 2003, the highest numbers of human cases. 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 participate in surveillance activities.

Table 3a provides a listing of the counties which participated during 2003, the numbers of sites and birds they maintained, the number of sera they submitted, the numbers of sentinels which seroconverted to EEE and to flavivirus (SLE/WN), the percent of exposed birds which seroconverted. Seroconversion dates are given in table 3b; confirmed seroconversions are indicated by "*"; presumptives are listed when a confirmation was not possible for that bird.

Figures 11, 12 and 13 depict the rate of seroconversions to EEE, SLE and WNV, respectively, by month, for the four state regions. Antibody response to WNV, peaked in September, a month

earlier than for SLE. In 2001, WN activity was low in the south and central regions and elevated in the north and panhandle regions of the state; 2002 saw low activity in the panhandle and increased activity in central and southern regions. In 2003, the seroconversion rates to WN were highest in the south and again in the panhandle regions. Rates increased earlier in the year and decreased sooner in the south than in the panhandle. Specific antibody to SLE was detected at a very low level. Historically, SLE has shown multiyear quiescent periods between widespread outbreaks.

Seroconversions to EEE occurred during most months of year, and peaked in July. Activity was highest in the panhandle and northern regions, as is traditional, and was significantly greater than the historical average rate. The annual regional seroconversion rates for SLE were well below each regional historical mean. This is the third year WNV has been detected in Florida, thus historical comparisons are not as useful as for agents with a long history in the state.

A total of 99,939 HAI, 2,423 ELISA and 3,692 SN tests were performed for arbovirus studies in 2003. This is a significant increase over past years (Table 4). Serological testing includes both the sentinel flock incidence studies, and wild bird sera submitted for prevalence studies.

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 (Brenda Brennan, Christy Voakes, Efe Akhigbe) 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 by year. Counties with sentinel chicken programs are indicated in **bold**.

county	2003 total #	2003 WN+	2002 total #	2002 WN+	2001 total #	2001 WN+
Alachua	85	13	190	21	294	64
Baker	2	0	0	0	5	2
Bay	149	108	230	7	446	86
Bradford	14	5	14	1	63	19
Brevard	1	0	93	3	307	3
Broward	34	9	137	1	185	14
Calhoun	14	8	8	2	16	8
Charlotte	2	0	23	1	69	8
Citrus	32	2	72	5	97	15
Clay	45	4	73	3	243	51
Collier	7	3	150	12	128	7
Columbia	14	6	37	2	51	29
Dade	269	25	203	2	397	21
Desoto	3	0	15	1	7	0
Dixie	14	0	18	1	35	9
Duval	21	2	42	0	537	101
Escambia	234	74	304	123	216	9
Flagler	1	0	20	6	74	2
Franklin	6	2	4	0	22	4
Gadsen	0	0	2	0	53	27
Gilchrist	7	3	22	5	38	11
Glades	1	1	7	0	19	1
Gulf	0	0	3	0	41	15
Hamilton	11	1	15	0	31	14
Hardee	0	0	3	2	2	1
Hendry	0	0	20	1	13	2
Hernando	68	1	102	7	129	8
Highlands	1	0	22	4	49	2
Hillsborough	51	3	92	8	157	2
Holmes	13	5	11	3	36	12
Indian River	5	0	5	1	12	0
Jackson	123	37	142	34	98	12
Jefferson	18	3	8	0	27	16
Lafayette	1	0	0	0	15	8
Lake	3	0	67	35	146	14
Lee	0	0	14	2	18	2
Leon	98	11	111	3	318	110
Levy	28	11	44	8	94	23
Liberty	8	2	0	0	17	9
Madison	29	4	24	0	35	10
Manatee	0	0	5	0	88	4
Marion	52	3	122	41	188	41
Martin	9	1	27	0	37	3
Monroe	52	2	59	0	165	18
Nassau	17	1	27	0	98	23
Okaloosa	193	58	144	5	410	25
Okeechobee	17	3	34	2	19	1
Orange	56	0	124	9	262	3
Osceola	18	1	25	0	46	2
Palm Beach	169	10	285	7	189	7
Pasco	22	1	86	12	249	21
Pinellas	47	1	45	6	207	1
Polk	11	0	26	0	61	5
Putnam	2	0	59	7	92	12
Santa Rosa	112	37	100	11	162	16
Sarasota	41	1	280	19	166	4
Seminole	39	0	88	5	203	4
St. Johns	0	0	1	0	33	0
St. Lucie	13	1	41	1	37	2
Sumter	3	0	33	6	35	1
Suwanee	4	1	7	0	102	48
Taylor	13	8	15	0	29	13
Union	12	2	10	1	32	8
Volusia	6	0	58	6	84	3
Wakulla	11	3	3	0	108	64
Walton	43	5	44	3	84	8
Washington	24	8	33	3	41	16
Unknown	0	0	2	0	6	2
Total	2398	489	4130	448	7773	1106

Table 2. Non-sentinel avian serum assayed during 2003.

County	# submitted	# EEE +	% EEE +	# WN +	% WN +
Alachua	19	3	16%	6	32%
Bay	1	0	0%	0	0%
Calhoun	1	0	0%	0	0%
Duval	5	0	0%	0	0%
Hillsborough	5	0	0%	1	20%
Indian River	163	0	0%	0	0%
Okaloosa	136	26	19%	5	4%
Orange	96	0	0%	3	3%
Osceola	18	3	17%	5	28%
Palm Beach	3	0	0%	1	33%
Santa Rosa	90	25	28%	3	3%
St. Lucie	35	0	0%	0	0%
Wakulla	1	0	0%	0	0%
Walton	287	33	11%	2	1%
Washington	412	26	6%	8	2%
Total	1272	116	9%	34	3%

Table 3a. ARBOVIRUS SURVEILLANCE REPORT: Sentinel flock activity by county-

County	# of Sites Monitored	# of Susceptibles Examined	# of Sera from Susceptibles Examined	# of Sentinels Seroconverting			Percent of Sentinels Seroconverting		
				EEE	SLE	WN	EEE	SLE	WN
Alachua	7	100	591	28	0	26	28.0%	0.0%	26.0%
Bay	3	85	641	15	0	47	17.6%	0.0%	53.3%
Brevard	10	82	1065	0	0	26	0.0%	0.0%	31.7%
Charlotte	9	106	764	0	4	47	0.0%	0.5%	44.3%
Citrus	6	57	508	2	0	25	3.5%	0.0%	43.9%
Collier	7	102	900	0	0	58	0.0%	0.0%	56.9%
Dade	6	86	897	0	1	17	0.0%	0.1%	19.8%
Duval	6	59	546	11	0	34	18.6%	0.0%	57.6%
Flagler	5	49	321	8	0	6	16.3%	0.0%	12.2%
Hendry/Glades	5	83	519	0	0	53	0.0%	0.0%	63.9%
Hillsborough	10	138	1358	12	1	41	8.7%	0.1%	29.7%
Indian River	10	217	2578	0	0	46	0.0%	0.0%	21.2%
Jefferson	4	77	490	3	0	19	3.9%	0.0%	24.7%
Jackson	4	30	182	7	0	18	23.3%	0.0%	60.0%
Lee	17	385	1499	0	2	114	0.0%	0.5%	29.6%
Leon	7	136	1188	20	0	45	14.7%	0.0%	33.1%
Manatee	12	168	2978	0	1	61	0.0%	0.0%	36.3%
Marion	5	66	518	2	0	20	3.0%	0.0%	30.3%
Martin	8	60	605	0	0	29	0.0%	0.0%	48.3%
Nassau	8	129	801	38	0	29	29.5%	0.0%	22.5%
Okeechobee	3	39	337	1	0	15	2.6%	0.0%	38.5%
Orange	19	317	5764	58	0	60	18.3%	0.0%	18.9%
Osceola	12	165	1707	8	0	25	4.8%	0.0%	15.2%
Palm Beach	10	215	1927	0	0	108	0.0%	0.0%	50.2%
Pasco	6	82	1274	5	0	6	6.1%	0.0%	7.3%
Pinellas	9	152	1513	4	0	15	2.6%	0.0%	9.9%
Putnam	8	121	505	24	0	39	19.8%	0.0%	32.2%
Santa Rosa	1	11	29	0	0	0	0.0%	0.0%	0.0%
Sarasota	11	162	3189	0	2	74	0.0%	0.1%	45.7%
Seminole	6	59	829	0	0	5	0.0%	0.0%	8.5%
St. Johns	10	116	1340	15	0	47	12.9%	0.0%	40.5%
St. Lucie	5	90	773	0	0	13	0.0%	0.0%	14.4%
Volusia	16	194	2447	6	0	18	3.1%	0.0%	9.3%
Walton	23	417	3827	46	0	160	11.0%	0.0%	38.4%
Washington	1	6	24	0	0	0	0.0%	0.0%	0.0%
Totals	289	4361	44434	313	10	1343			

Table 3b. ARBOVIRUS SURVEILLANCE REPORT: Sentinel flock activity by county

County	Week of Sero Conversion (* Indicates Confirmed)
Alachua	**EEE**: 4/14(1*), 4/15(1*), , 4/21(2*), 5/6(1*), 5/13(2*), 5/27(4*), 5/28(2*), 6/2(3*), 6/30(1*), 7/8(1*), 7/14(4*), 7/21(2*), 8/11(1*), 9/8(1), 9/16(1*,1); **WN**: 8/11(4*), 8/18(5*), 9/8(5*), 9/16(2)*, 9/23(1*), 10/13(4*), 10/20(4*), 10/27(1*)
Bay	**EEE**: 5/6(1*), 7/8(4*), 7/15(4*), 7/22(3*), 7/2(1*), 8/4(2*); **WN**: 7/15(1*), 7/22(2*), 8/12(3*), 8/19(1*,1*n), 8/19(8*,1*n), 9/2(1*), 9/9(5*n), 9/16(10*), 10/7(3*), 10/21(5*), 10/28(2*), 11/18(1*,3*n)
Brevard	**WN**: 4/24(3*), 5/29(1*), 7/31(1*), 8/7(1*), 8/21(2*), 8/22(2*), 9/4(1*), 9/12(3*), 9/19(2*), 10/10(1*), 10/17(1*) 10/23(1*), 10/30(1*), 11/6(1*), 11/13(5*),
Charlotte	**SLE**: 10/3(1*), 10/17(1*), 10/31(1*), 12/12(1*); **WN**: 5/30(1*), 6/9(1*), 7/25(1*), 8/1(1*), 8/8(4*), 8/15(4*), 8/22(8*), 8/29(2*), 9/5(2*), 9/12(2*), 9/19(9*), 9/26(3*), 10/17(2*), 10/24(2*), 11/7(1*n), 12/5(1*n), 12/12(3*)
Citrus	**EEE**: 7/21(1*), 12/1(1*); **WN**: 8/11(1*), 8/18(3*), 8/25(4*), 9/8(6*), 9/29(1*), 10/6(3*), 10/10(1*), 10/13(2*), 10/27(1*), 11/10(2*), 12/8(1*)
Collier	**WN**: 5/27(1*), 6/2(2*), 6/9(2*), 6/23(1*), 6/30(1*), 7/7(2*), 7/14(3*), 7/21(3*), 7/28(3*), 8/4(3*), 8/11(4*), 8/19(2*), 8/25(4*), 9/2(2*), 9/8(2*), 9/15(1*), 9/22(1*), 9/29(1*), 9/30(1*), 10/6(1*), 10/13(3*), 10/20(1*), 10/27(4*), 11/3(1*), 11/10(2*), 11/17(4*), 12/8(2*), 12/31(1*)
Dade	**SLE**: 10/28(1*); **WN**: 7/15(1*), 7/21(1*), 7/29(1*), 8/4(2*), 8/11(1*), 8/18(2*), 8/25(2*), 9/2(3*), 9/16(2*), 9/29(2*)
Duval	**EEE**: 5/16(1*), 5/27(1*), 6/9(1*), 6/10(2*), 6/23(1*), 7/22(2*), 7/29(1*), 12/1(2*); **WN**: 7/22(1*), 8/12(1*), 8/19(6*), 9/2(1*), 9/8(1*), 9/15(10*), 9/22(5*), 9/29(2*), 10/27(1*), 11/13(1*), 11/17(4*), 12/8(1*)
Flagler	**EEE**:4/7(1*), 6/2(1*), 6/9(1*), 6/16(2*), 6/30(1^{n}), 7/14(1*), 7/21(1*); **WN**: 9/29(1*), 10/9(1*), 10/20(1*), 10/27(1*), 11/7(1*), 11/12(1*)
Hendry/Glades	**WN**: 6/2(2*), 7/11(1*), 7/25(1*), 7/28(1*), 8/1(1*), 8/8(4*), 8/11(5*), 8/15(2*), 8/22(1*), 8/25(1*), 9/8(6*), 9/12(4*), 9/22(9*, 2*n), 9/26(2*), 10/17(1*), 10/21(3*), 10/24(3*), 11/5(2*), 11/7(1*), 12/12(1*)
Hillsborough	**EEE**: 3/17(1*), 3/31(1*), 4/21(1*), 5/19(1*), 5/27(1*), 6/2(2*), 6/9(4*), 6/16(1*); **SLE**: 11/10(1*); **WN**: 7/21(1*), 8/4(1*), 8/11(4*), 8/18(2*), 8/25(9*), 9/8(1*), 9/15(3*), 9/29(6*), 10/6(3*), 10/13(2*), 11/3(1*), 11/10(1*), 11/18(5*), 11/25(1*), 12/9(1*)
Indian River	**WN**: 1/9(1*), 5/1(3*), 5/8(1*), 6/12(3*), 6/19(2*), 8/7(1*), 8/15(2*), 8/21(7*), 8/28(2*), 9/11(5*), 10/2(1*), 10/9(2*), 10/16(4*), 10/23(1*), 10/30(1*), 11/6(6*), 11/20(3*), 12/30(1*)
Jackson	**EEE**: 10/30(1*^{n}, 1^{n}), 11/13(1*); **WN**: 8/12(1*), 9/2(2*), 9/16(1*), 9/18(2*), 9/23(1*), 10/7(1*), 10/9(1*,1*^{n}), 10/28(1*), 10/30(1*^{n}), 11/18(4*), 11/20(3*)
Jefferson	**EEE**: 6/30(1*^{n}), 7/27(4*), 8/17(1^{n}), 10/19(1*); **WN**: 8/10(1*), 8/17(2*), 9/14(4*), 9/20(4*), 9/28(1*), 10/5(2*), 10/12(1*), 10/19(2*), 10/26(1*)
Lee	**SLE**: 10/31(1*n), 12/8(1*); **WN**: 1/9(4*), 2/12(1*), 4/8(1*), 4/29(1*), 6/17(1*), 7/8(3*), 7/14(4*), 7/21(3*), 7/29(9*), 8/4(12*), 8/10(9*), 8/18(6*), 8/25(6*), 8/26(6*), 9/8(6*), 9/9(10*), 9/16(3*), 9/23(12*), 10/6(6*), 10/13(1*, 1*n), 11/4(1*), 11/11(1*), 11/17(3*), 12/2(1*), 12/16(1*)

County	Week of Sero Conversion (* Indicates Confirmed)
Leon	**EEE**: 7/7(2*), 7/14(1*), 7/18(2*), 7/25(2*), 8/1(3*), 8/8(1*), 8/22(5*), 8/29(1*), 10/31(1*), 11/13(1*), 11/21(1*); **WN**: 7/25(1*), 8/1(1*), 8/15(1*), 9/4(6*), 9/12(4*), 9/18(1*), 9/19(3*), 9/25(4*), 10/3(5*), 10/10(2*), 10/13(1*), 10/17(3*), 10/23(6*), 10/31(1*, 1*n), 11/7(2*), 11/20(1*), 11/21(1*), 12/1(1*)
Manatee	**SLE**: 11/4(1*); **WN**: 1/17(1*), 3/10(1*), 5/2(1*), 7/11(1*), 8/1(2*), 8/8(7*), 8/22(10*), 8/29(6*), 9/5(2*), 9/8(5*), 9/10(3*), 9/11(1*), 9/15(1*), 9/25(2*), 10/8(1*), 10/20(2*), 10/22(5*), 10/28(1*), 11/3(2*), 11/5(2*), 11/12(1*), 11/19(2*), 12/8(1*), 12/15(1*)
Marion	**EEE**: 6/13(1^{n}*), 7/27(1*); **WN**: 8/17(1*), 8/30(2*), 9/21(1*), 9/28(1*), 10/18(4*), 10/25(2*), 10/26(1*), 11/1(3*), 11/9(1*), 11/16(1*), 12/6(2*), 12/7(1*)
Martin	**WN**: 7/7(1*), 7/18(2*), 7/28(1*), 8/1(1*), 8/15(5*), 8/25(1*), 9/5(2*), 9/12(10*), 9/22(1*), 9/26(1*), 10/3(1*), 10/6(1*), 10/17(1*n), 11/7(1*)
Nassau	**EEE**: 5/25(2*), 6/9(5*), 6/23(3*), 7/7(1*), 7/14(18*), 7/21(1*), 7/28(2*), 8/4(2*,1), 8/11(1*), 8/18(2*); **WN**: 6/30(1*), 7/21(1*), 7/28(2*), 8/11(1*), 8/18(1*), 8/25(2*), 9/1(4*), 9/8(11*), 9/15(5*), 9/22(1*)
Okeechobee	**EEE**: 8/25(1*); **WN**: 8/18(4*), 9/2(1*), 9/8(3*), 9/15(1*), 9/22(1*), 10/6(1*), 10/13(1*), 11/3(1*), 12/8(2*)
Orange	**EEE**: 3/27(2*), 4/17(3*), 4/28(1*), 5/1(1*), 5/8(3*), 5/12(1*), 5/15(5*), 5/22(4*), 5/27(1*), 5/30(4*), 6/5(1*), 6/16(1*,1), 6/19(1*), 6/26(4*), 6/30(1*), 7/2(1*), 7/3(1*), 7/10(10*), 7/14(8), 8/4(1*,1), 8/7(1*), 8/11(1); **WN**: 4/28(1*), 6/19(1*), 7/4(1*), 7/14(1*), 7/28(1*), 8/7(4*), 8/11(1*), 8/15(3*), 8/18(6*), 8/22(3*), 9/8(3*), 9/15(1*), 9/25(1*), 9/29(3*,1*^{n}), 10/6(2*), 10/10(3*), 10/13(3*), 10/15(1*), 10/20(1*), 10/23(1*), 10/27(3*), 11/10(2*), 11/17(2*), 11/24(1), 11/26(1), 12/1(1*), 12/5(3*), 12/8(3*), 12/15(2*)
Osceola	**EEE**: 4/15(1), 4/29(1*), 5/28(1*), 7/20(2*), 7/31(1*), 9/30(1*^{n}), 11/10(1*); **WN**: 2/4(1*), 3/18(1*), 8/17(3*), 8/15(1*), 8/22(1*), 9/2(3*), 9/14(1*), 9/16(2*), 9/21(1*), 9/25(1*), 9/30(1*), 10/12(1*), 10/26(2*), 11/3(1*), 11/18(1*), 11/23(1*), 12/9(2*,1*n)
Palm Beach	**WN**: 7/14(1*), 7/28(4*), 8/4(5*), 8/11(5*, 4*n), 8/18(5*, 2*n), 8/25(9*, 4*n), 9/2(2*,1*n), 9/8(1*,2*n), 9/15(13*, 1*n), 9/22(3*), 9/28(1*, 6*n), 9/29(10*), 10/6(2*), 10/13(7*), 10/20(8*,1*n), 10/27(4*, 1*n), 11/10(3*), 11/17(1*), 12/8(2*)
Pasco	**EEE**: 3/3(1*), 4/22(1*), 6/9(2*), 6/23(1*); **WN**: 1/13(1*), 8/4(1*), 10/13(1*), 10/21(1*), 10/27(2*)
Pinellas	**EEE**: 3/13(1*), 5/19(1), 6/2(1*), 8/4(1); **WN**: 1/13(1*), 6/16(1*), 7/28(2*), 8/18(1*), 8/25(1*), 9/2(5*), 9/8(2*), 9/15(2*)
Putnam	**EEE**: 4/24(1*), 4/25(1*), 5/1(1*), 5/16(1*); 5/29(2*), 5/30(1*), 6/6(1*), 6/13(1*), 6/19(2*), 6/26(1*n), 7/3(1*), 7/10(3*,1), 7/17(3*), 7/24(1*), 8/8(1*), 9/19(2*); **WN**: 4/25(2*), 5/15(1*), 5/30(1*), 7/2(1*), 7/18(1*n), 8/14(6*), 8/15(2*), 8/21(2*), 8/28(3*), 9/8(3*), 9/11(3*), 9/18(5*), 9/25(2*), 10/2(3*), 10/16(2*), 10/22(1*), 10/30(1*)
Santa Rosa	none
Sarasota	**SLE**: 10/6(1*), 11/17(1*); **WN**: 1/13(1*), 3/3(1*), 7/14(1*), 8/4(5*), 8/11(6*), 8/18(1*), 8/25(9*), 9/2(5*), 9/8(5*), 9/22(8*), 9/29(4*), 10/13(2*), 10/20(4*), 10/27(3*), 11/3(2*), 11/17(7*), 11/24(3*), 12/1(1*), 12/8(2*), 12/15(2*), 12/29(2*)

County	Week of Sero Conversion (* Indicates Confirmed)
Seminole	**WN**: 8/7(1*), 8/8(1*), 8/14(2*), 10/2(1*)
St. Johns	**EEE**: 6/9(1*), 6/16(3*), 6/23(1*), 7/7(1*), 7/14(2*), 7/28(1*), 8/18(1), 9/29(1*n), 10/20(1*), 11/4(1*n), 11/17(2*); **WN**: 6/16(1*), 7/8(1*n), 7/21(1*), 7/28(2*), 8/4(6*), 8/11(3*), 8/18(1*, 3*n), 8/25(3*), 9/2(1*), 9/15(2*), 9/22(2*), 9/29(1*), 10/6(4*), 10/13(2*), 10/20(2*), 10/27(4*), 11/4(1*), 11/17(2*n,1*), 12/1(1*), 12/8(3*)
St. Lucie	**WN**: 7/3(1*), 8/8(2*), 8/14(2*), 9/5(3*), 9/11(1*), 9/18(1*), 9/25(1*), 10/9(1*), 10/16(1*)
Volusia	**EEE**: 5/12(1), 5/19(1*), 5/27(1*), 7/14(1), 7/21(2*); **WN**: 1/13(2*), 1/21(1*), 3/3(1*), 7/14(1*), 7/21(1*), 8/4(1*), 8/11(1*), 8/18(2*), 8/25(1*), 9/29(1*), 10/6(1*), 10/13(1*), 11/10(2*), 11/17(1*), 12/15(1*)
Walton	**EEE**: 3/24(2*), 5/27(2*), 6/2(1*), 6/9(1*), 6/23(1*), 6/30(5*), 7/7(3*), 7/14(4*), 7/21(1*,2), 7/23(1*), 7/28(5*), 8/18(1*), 9/11(1*), 9/22(3*n), 10/23(1*), 10/31(2*), 11/13(1*), 11/17(1*n,1*), 11/24(1), 12/16(6*); **WN**: 6/20(1*), 7/7(1*), 7/14(3*), 7/21(2*), 7/28(1*), 7/30(1*), 8/3(3*), 8/6(1*), 8/7(4*), 8/10(1*), 8/18(1*n), 8/25(4*, 2*n), 8/28(4*), 9/2(3*,2*n), 9/3(1*), 9/8(9*), 9/9(1*), 9/10(1*), 9/11(1*), 9/12(1*), 9/15(4*), 9/22(1*,3*n), 9/23(5*), 9/24(1*n), 9/25(7*), 9/26(1*n), 9/29(5*,2n), 10/1(1*), 10/3(2*), 10/6(4*), 10/15(4*,3*n), 10/20(6*), 10/21(4*), 10/22(4*), 10/23(1*), 10/27(4*), 10/30(2*,1*n), 10/31(2*), 11/3(8*), 11/5(3*), 11/6(1*), 11/7(1*), 11/11(2*), 11/14(4*,1*n), 11/19(2*), 11/20(1*), 11/21(1*), 11/24(2*), 12/1(1*), 12/2(2*), 12/4(1*,1*n), 12/8(4*), 12/10(3*,2*n), 12/11(2*), 12/15(3*)
Washington	none

Table 4. Number of arbovirus assays performed 2000-2003

Assay	2003	2002	2001	2000
Serology				
HAI	99,939	74,075	65,458	41,698
IgM Elisa	2,423	1,376	633	440
SN	3,692	2,343	924	839
Virology				
Animal tissue	2,320	4,009	7,773	19
Mosquito pools	6,292	4,130	1,378	160

Figure 1: Animal tissue submissions per week for West Nile virus detection

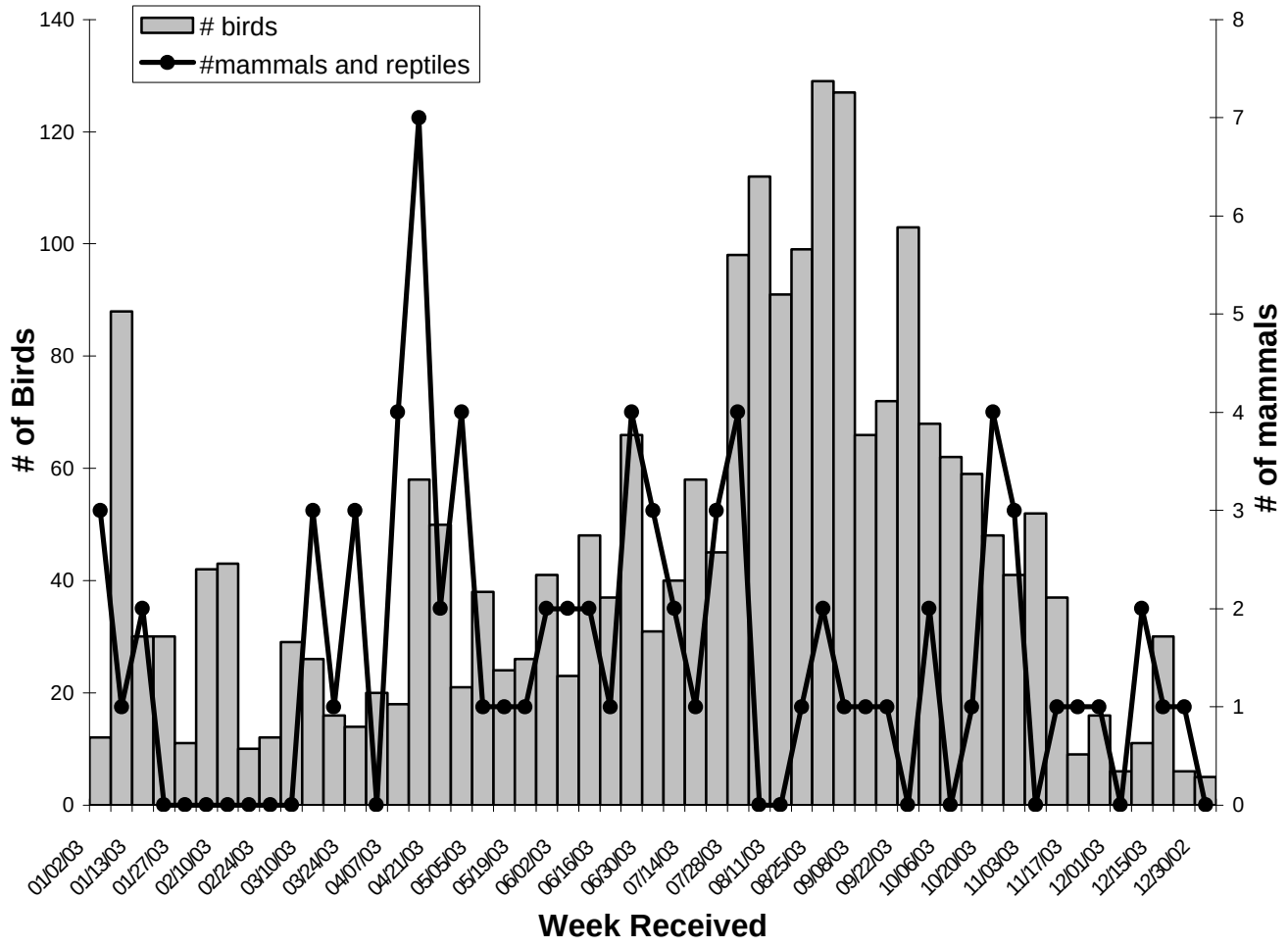

Figure 2a. Numbers of sentinel chicken seroconversions to EEE by month: 1988 to 2003

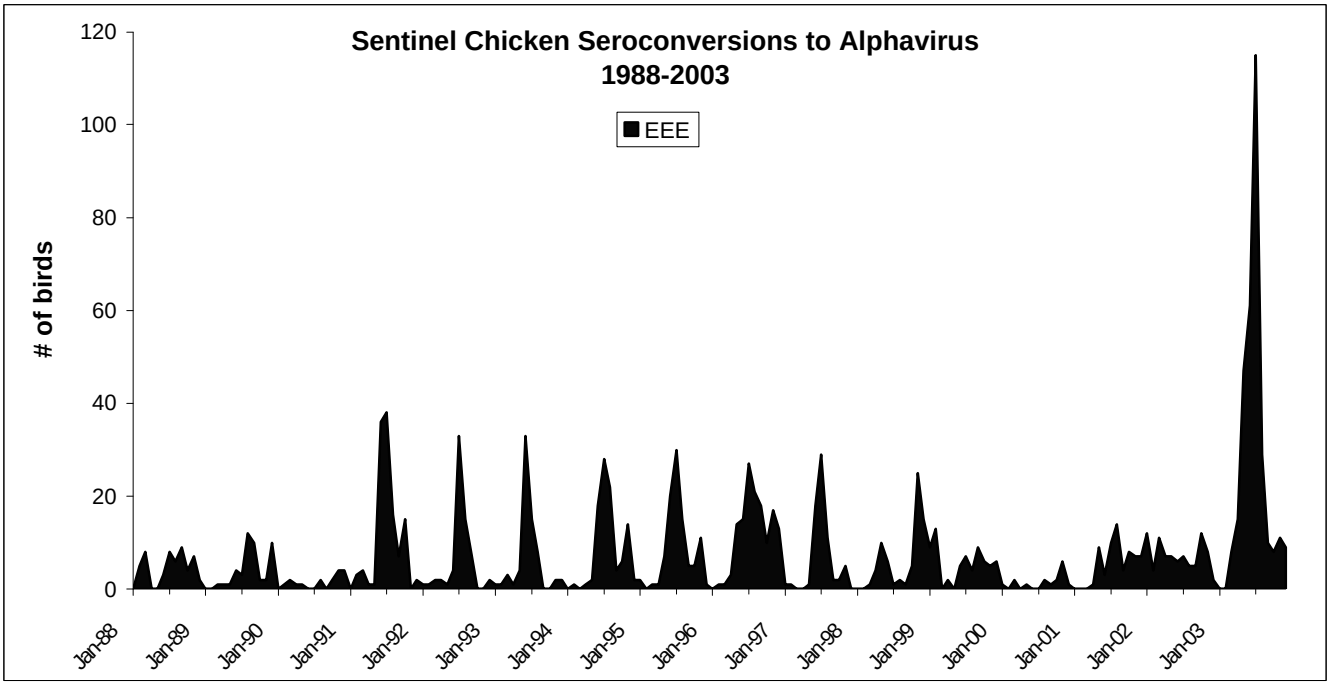

Figure 2b. Rate of sentinel Chicken seroconversion to EEE by month: 1988 to 2003

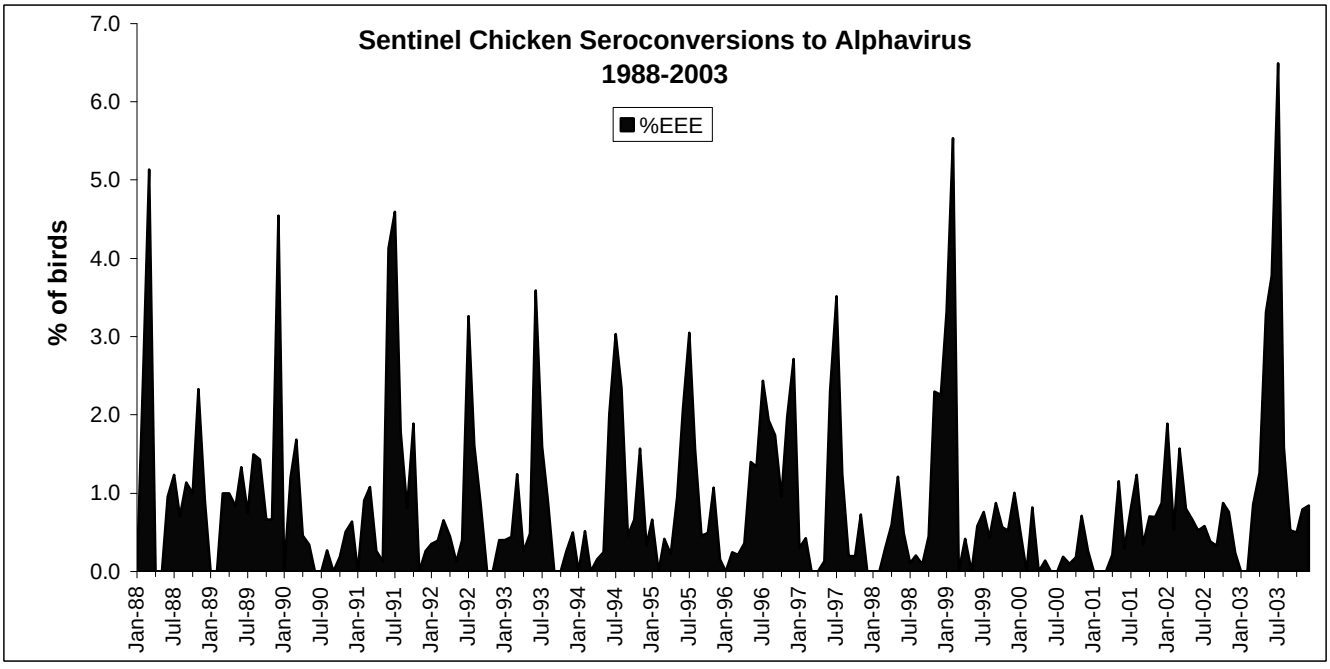

Figure 3a. Numbers of sentinel chicken seroconversions to Flavivirus (SLE/WN) by month: 1988 to 2003

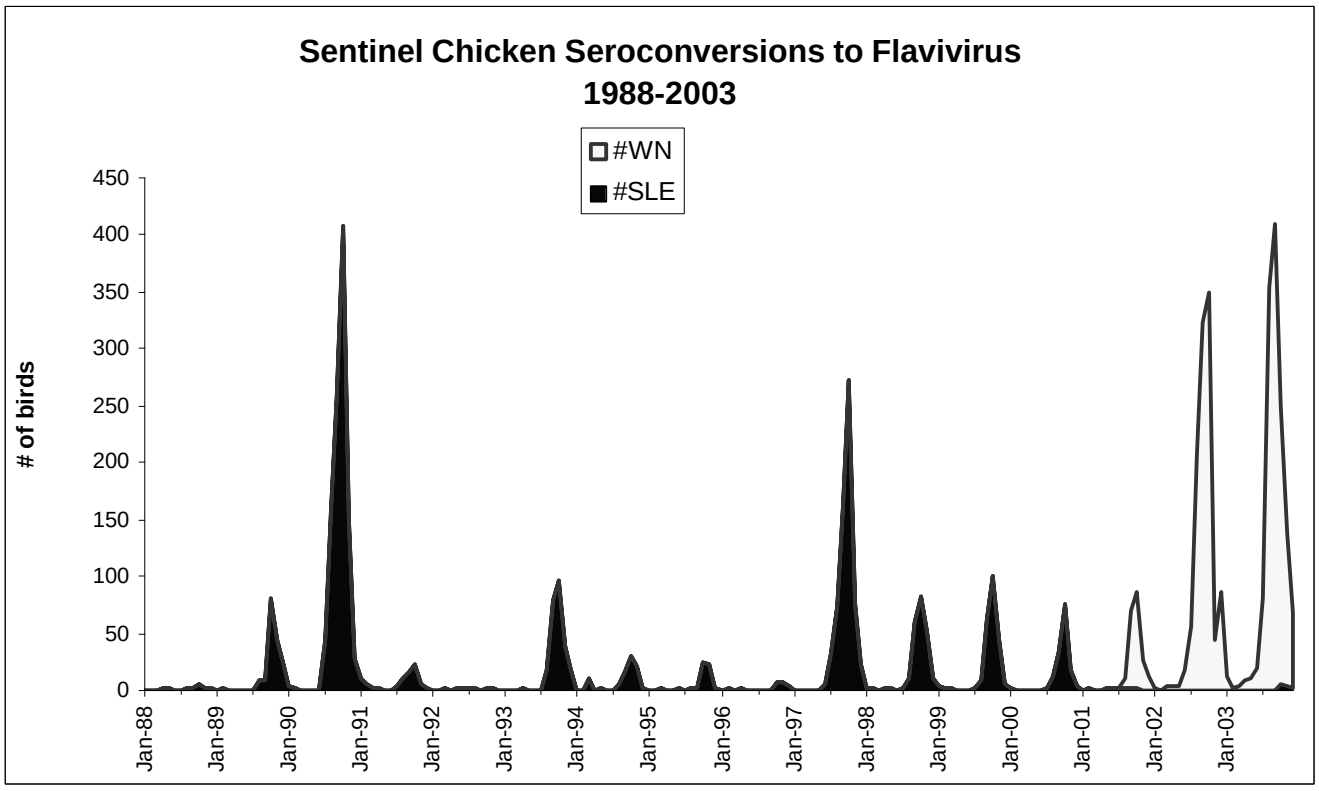

Figure 3b. Rate of sentinel Chicken seroconversion to Flavivirus (SLE/WN) by month: 1988 to 2003

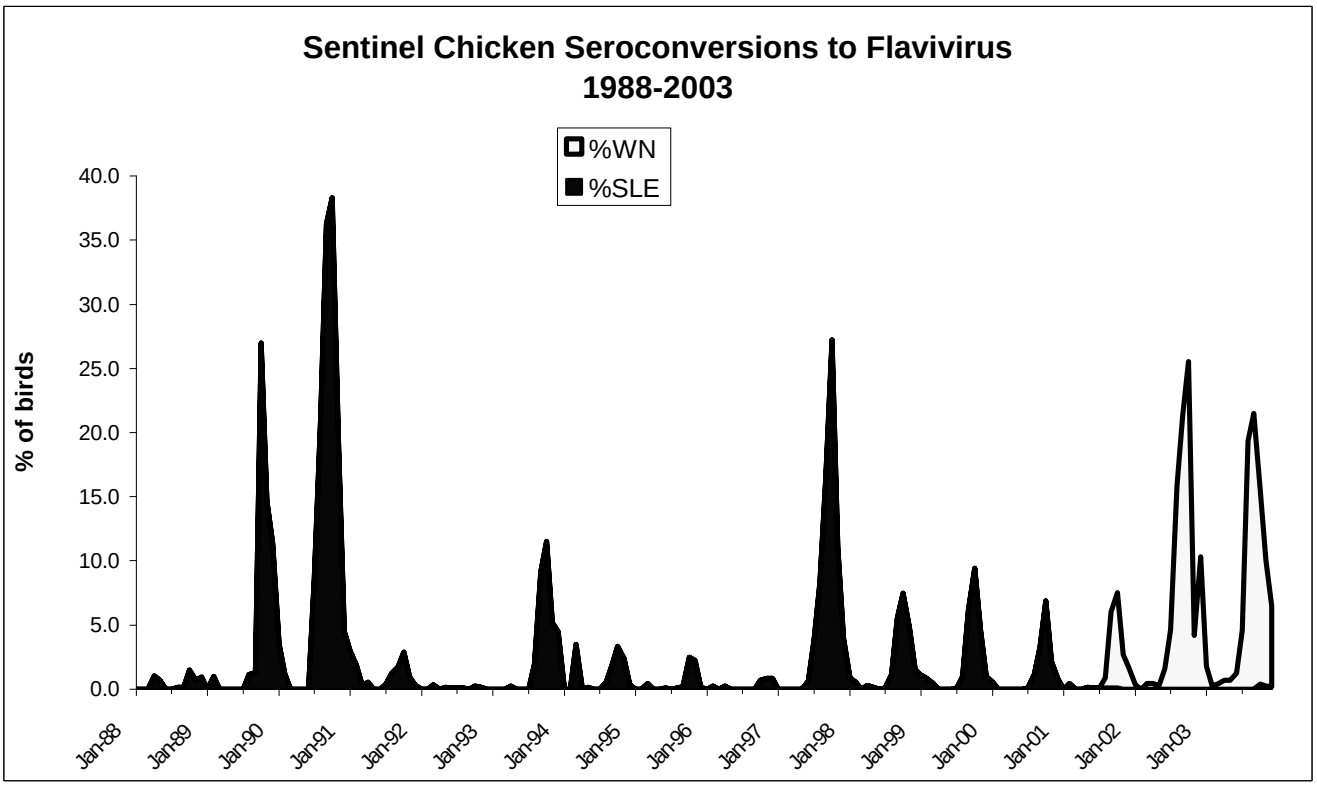

Figure 4a. Number of sentinels by region by month, 2003

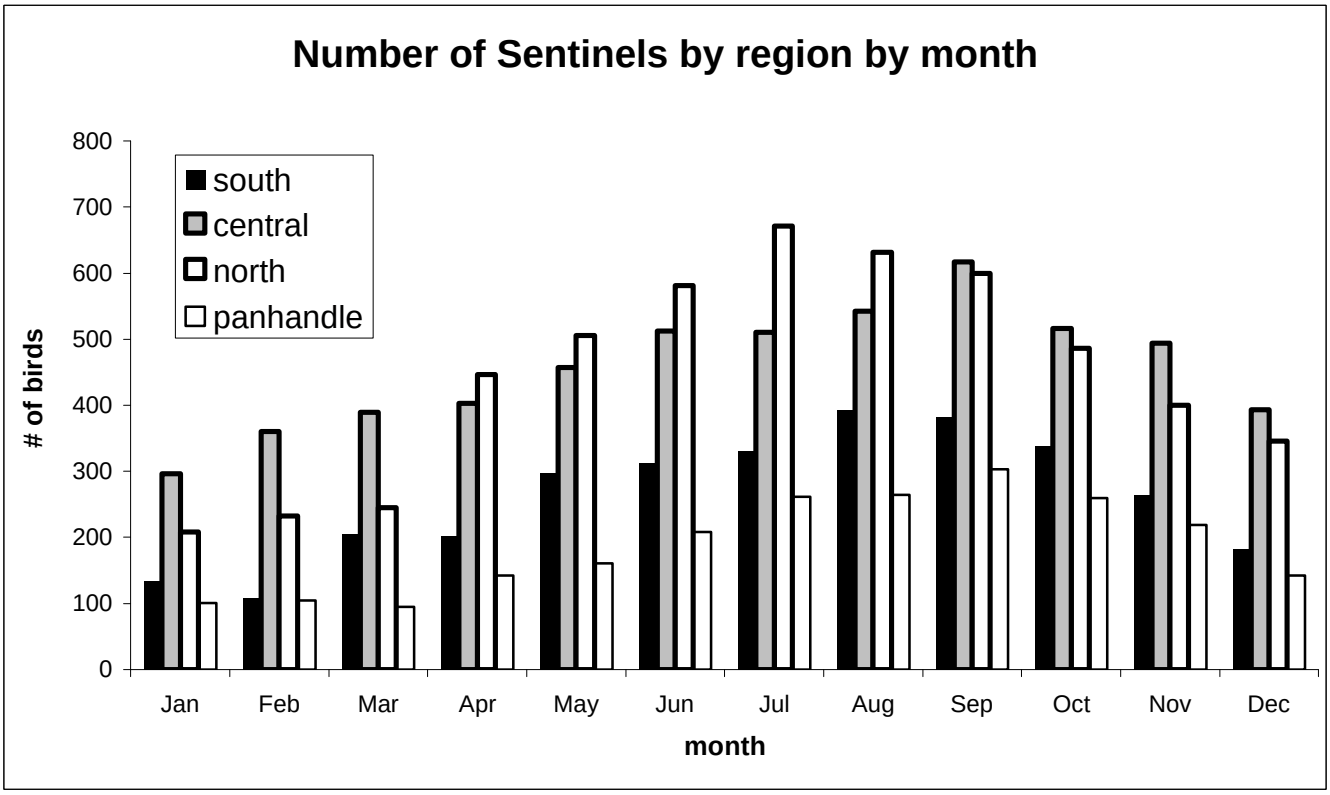

Figure 4b. Numbers of sentinel sera submitted by region by month, 2003

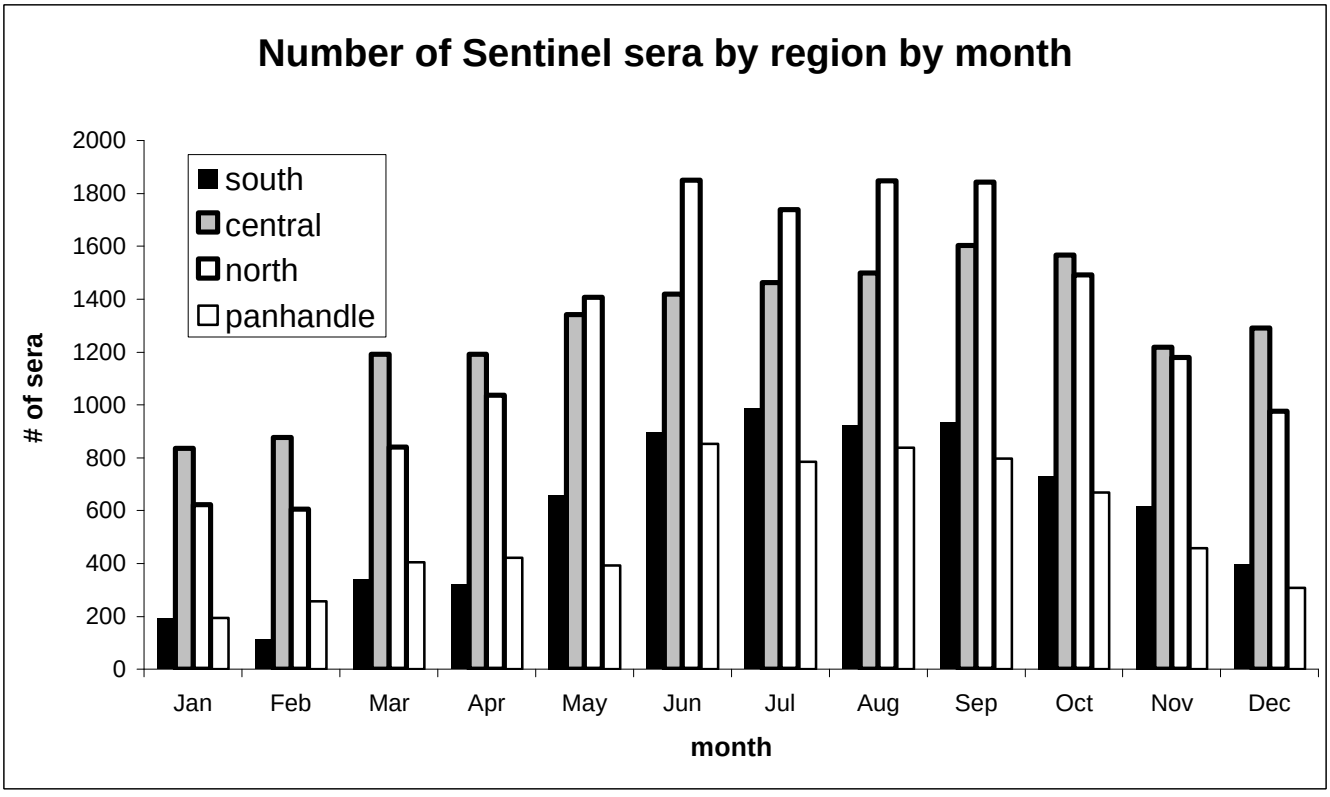

Figure 5. Number of sentinel sites by county, 2003.

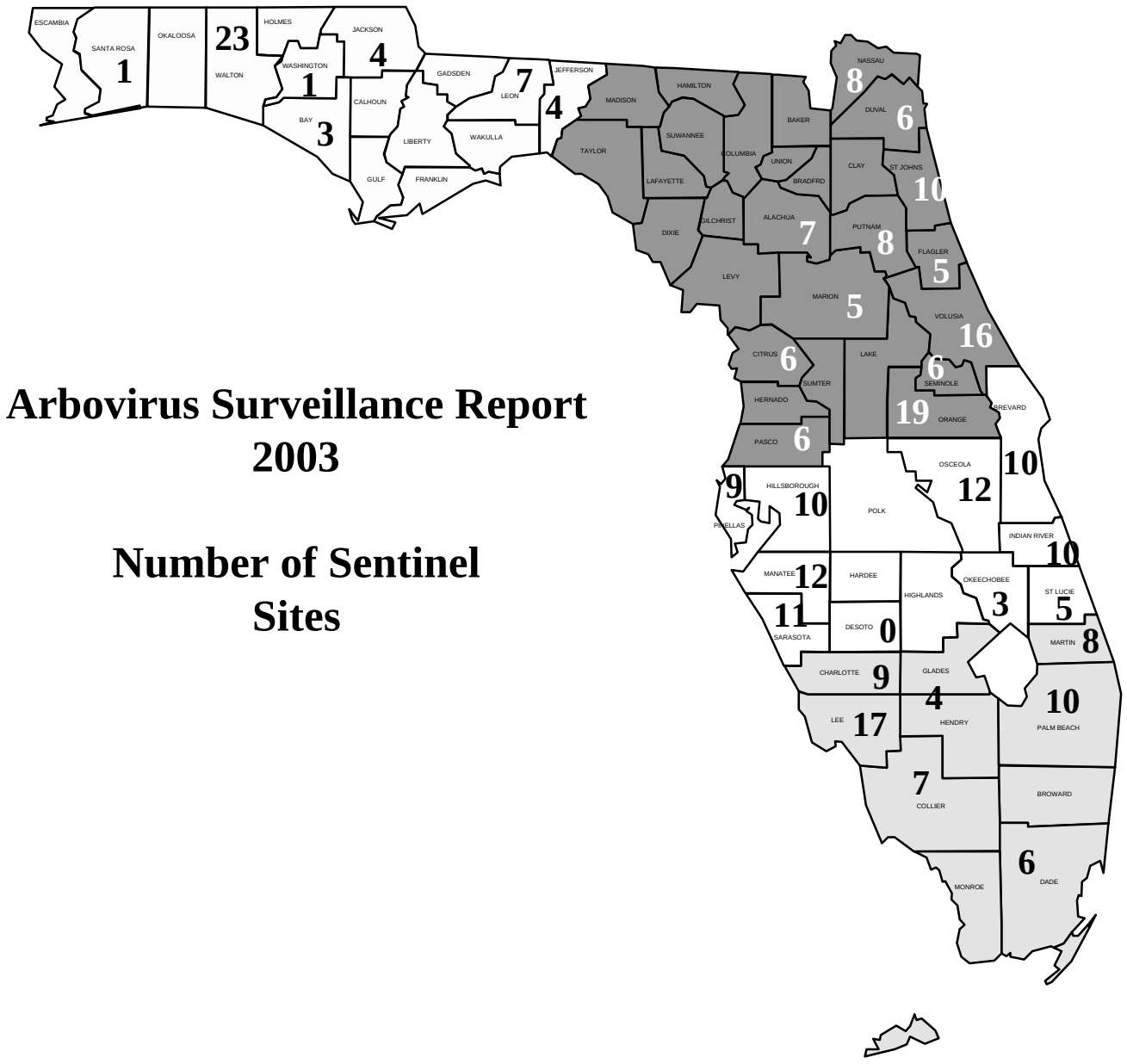

Figure 6. Number of sentinel birds by county, 2003.

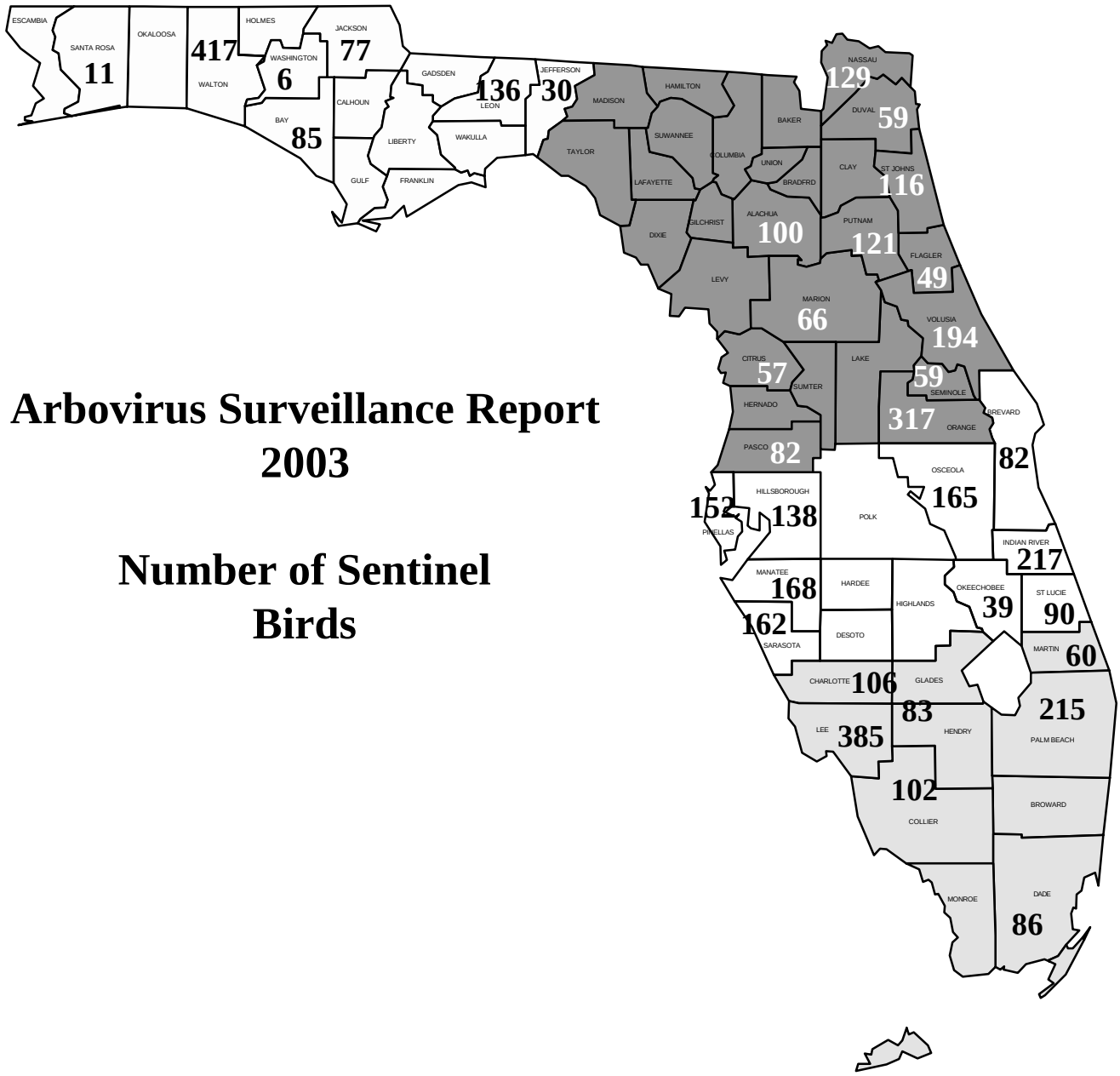

Figure 7.Number of sentinel sera by county, 2003.

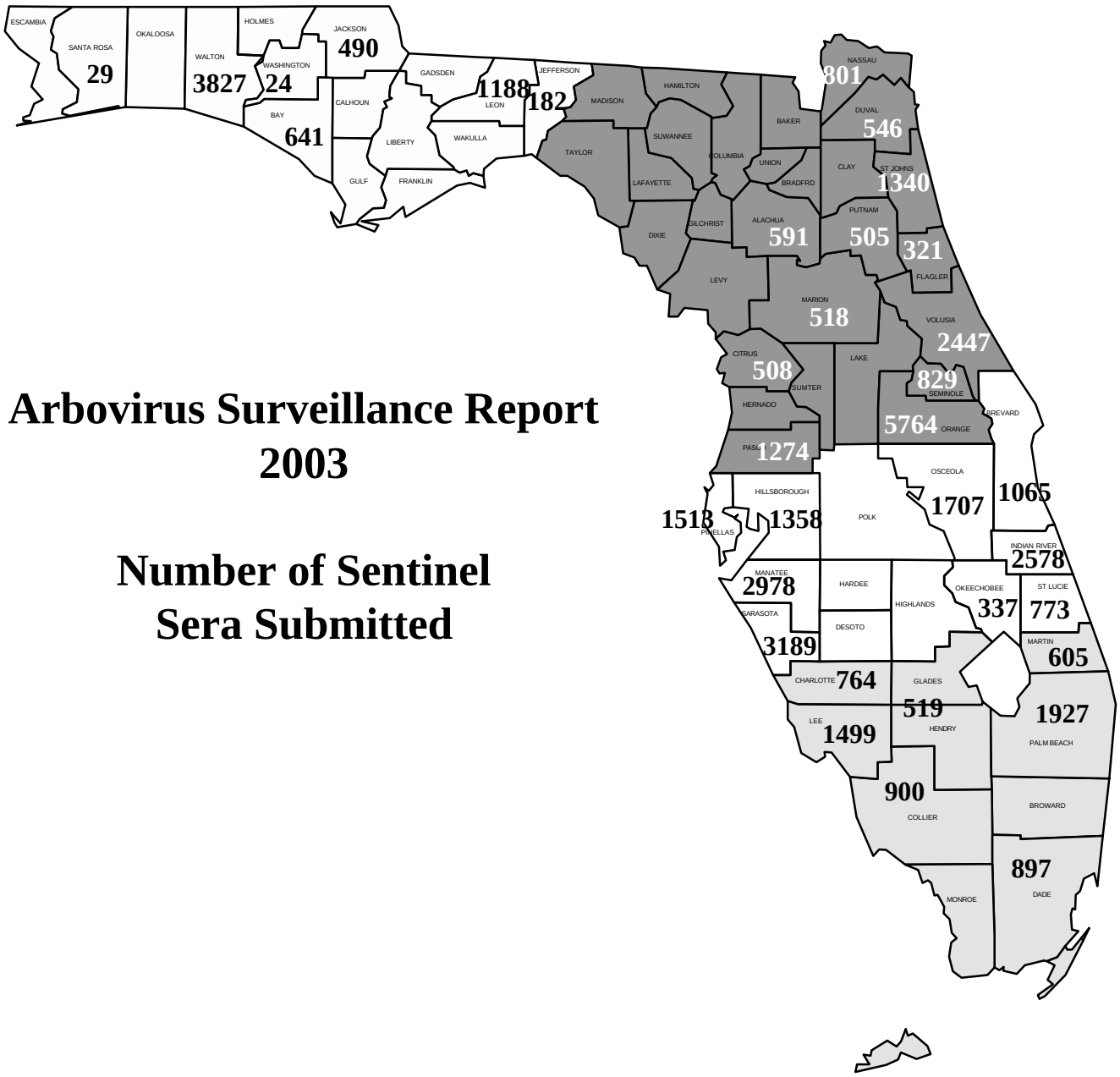

Figure 8. Number of sentinel seroconversions to EEE virus by county, 2003

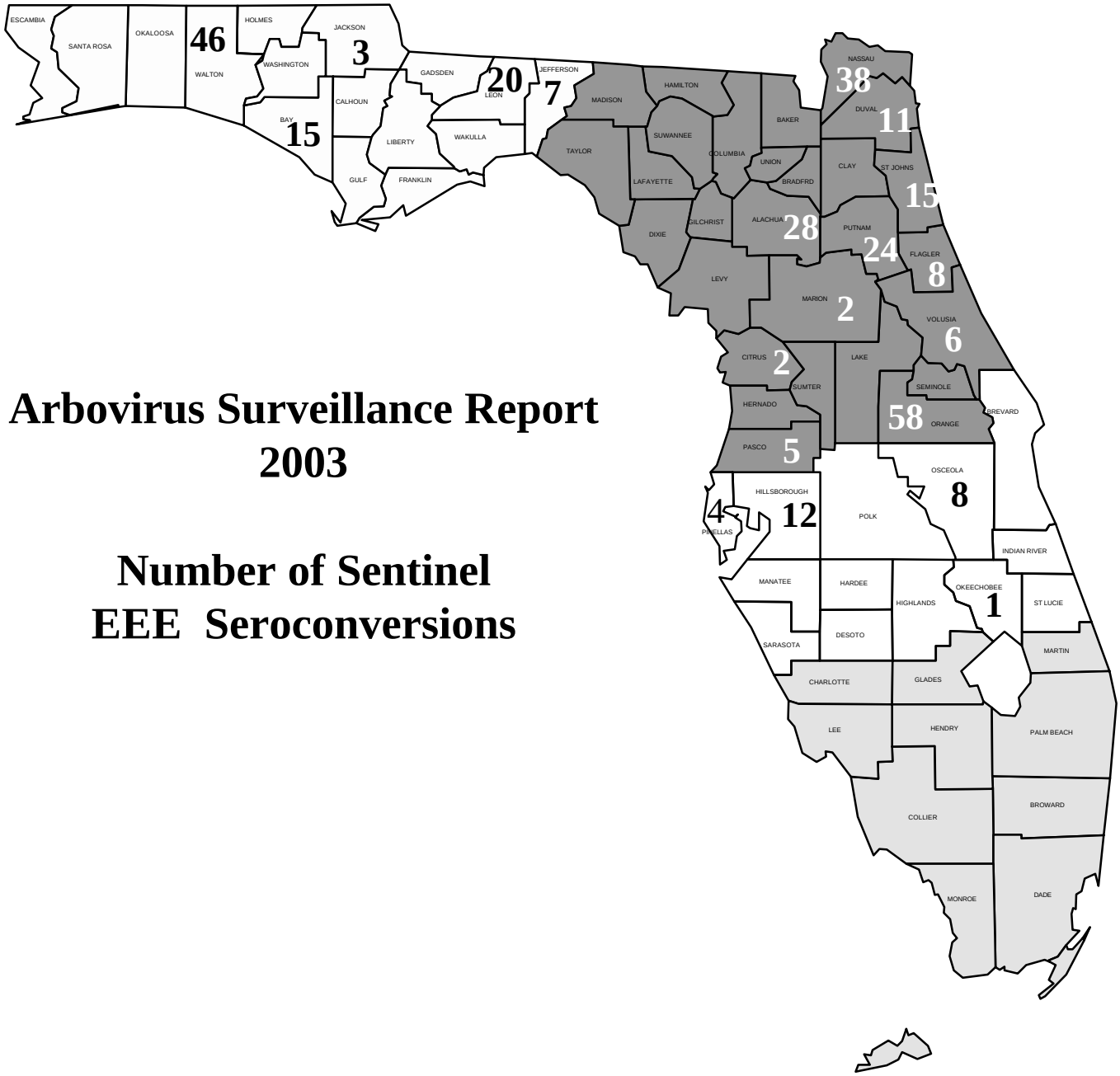

Figure 9. Number of sentinel seroconversions to SLE virus by county, 2003

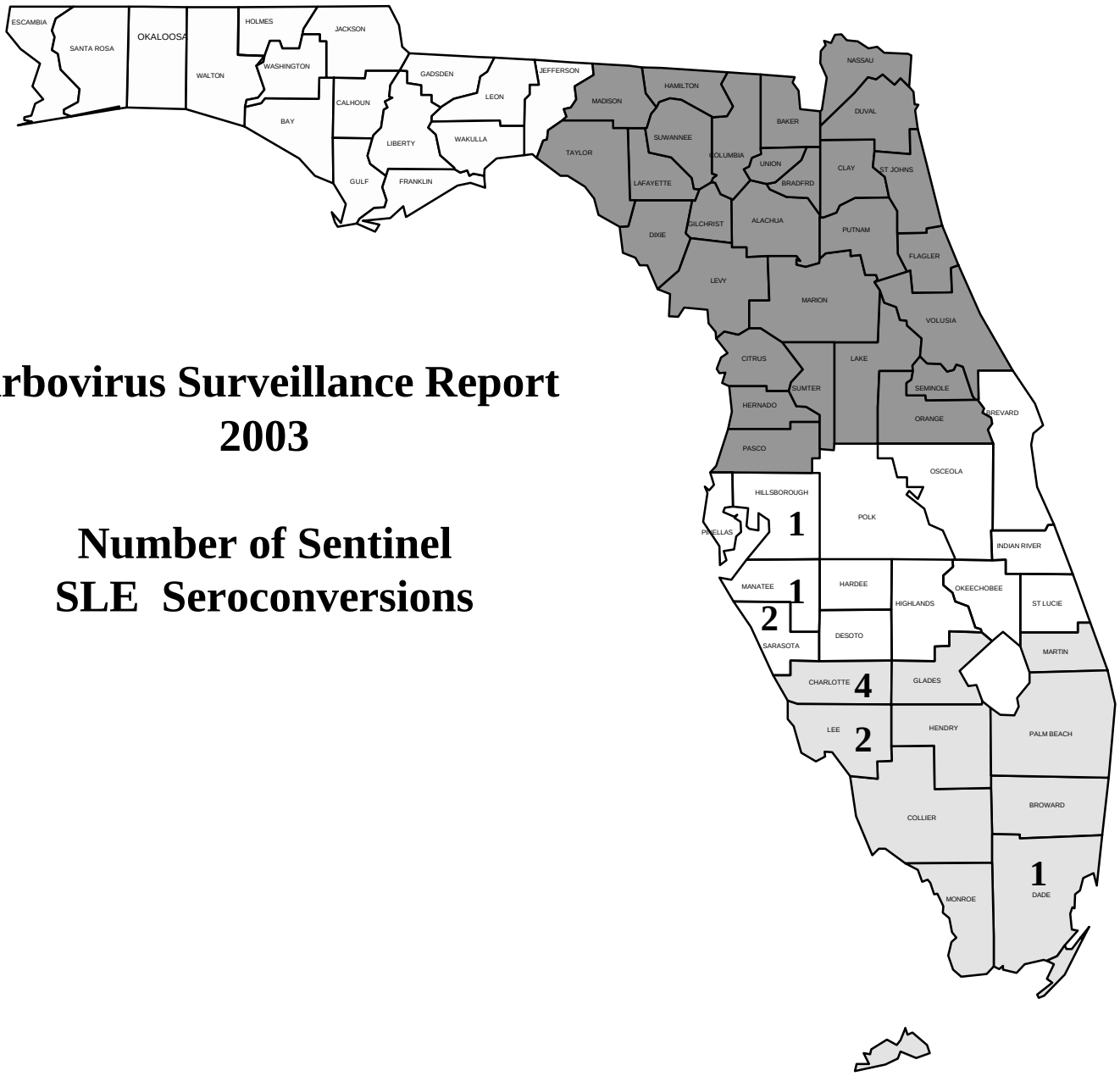

Figure 10. Number of sentinel seroconversions to WN virus by county, 2003

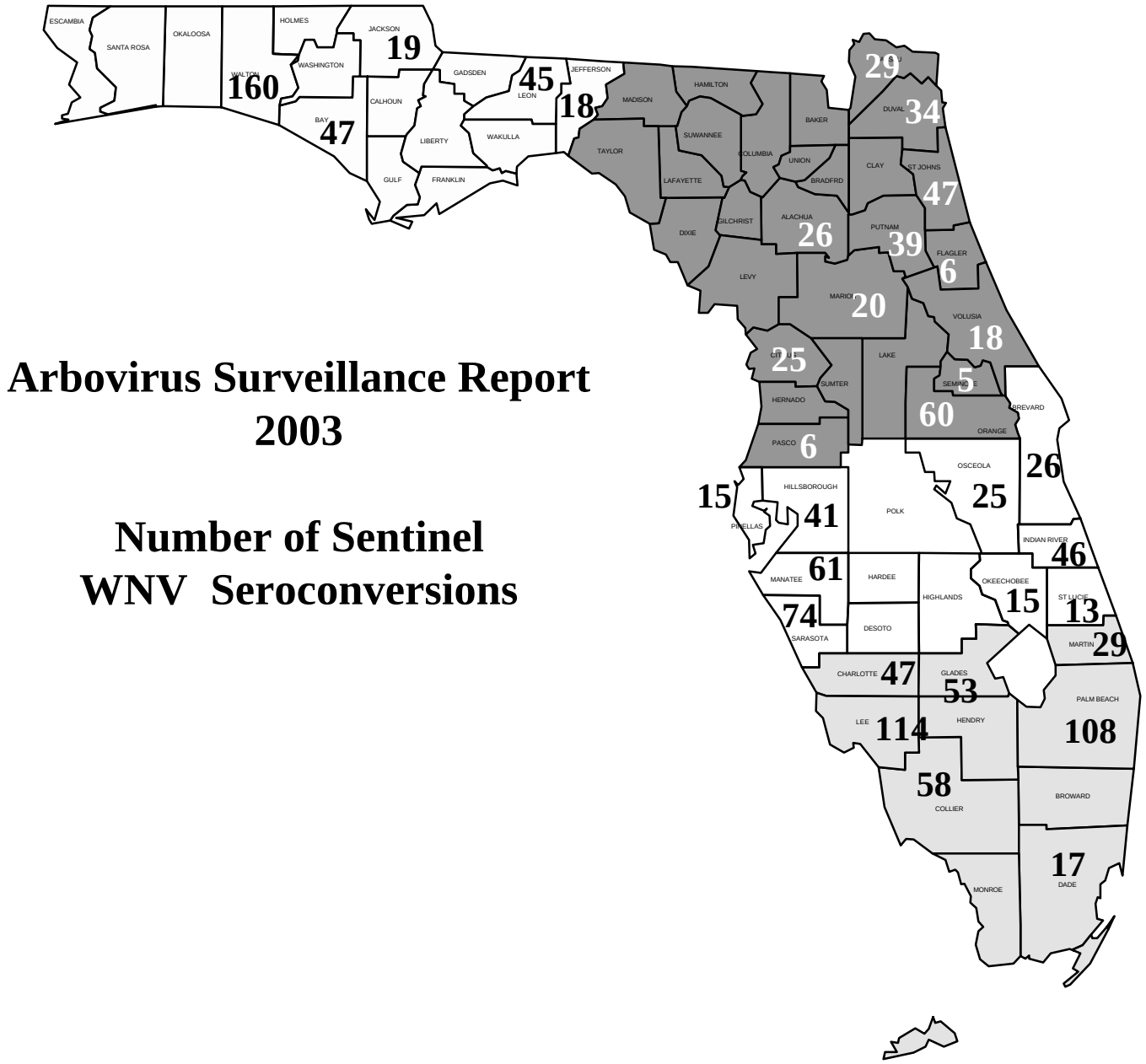

Figure 13. Rate of sentinel seroconversions to EEE virus by region by month, 2003.
"State" is the statewide rate for that month for 2003; "Avg. historical" is the average statewide rate from 1988 through 2003.

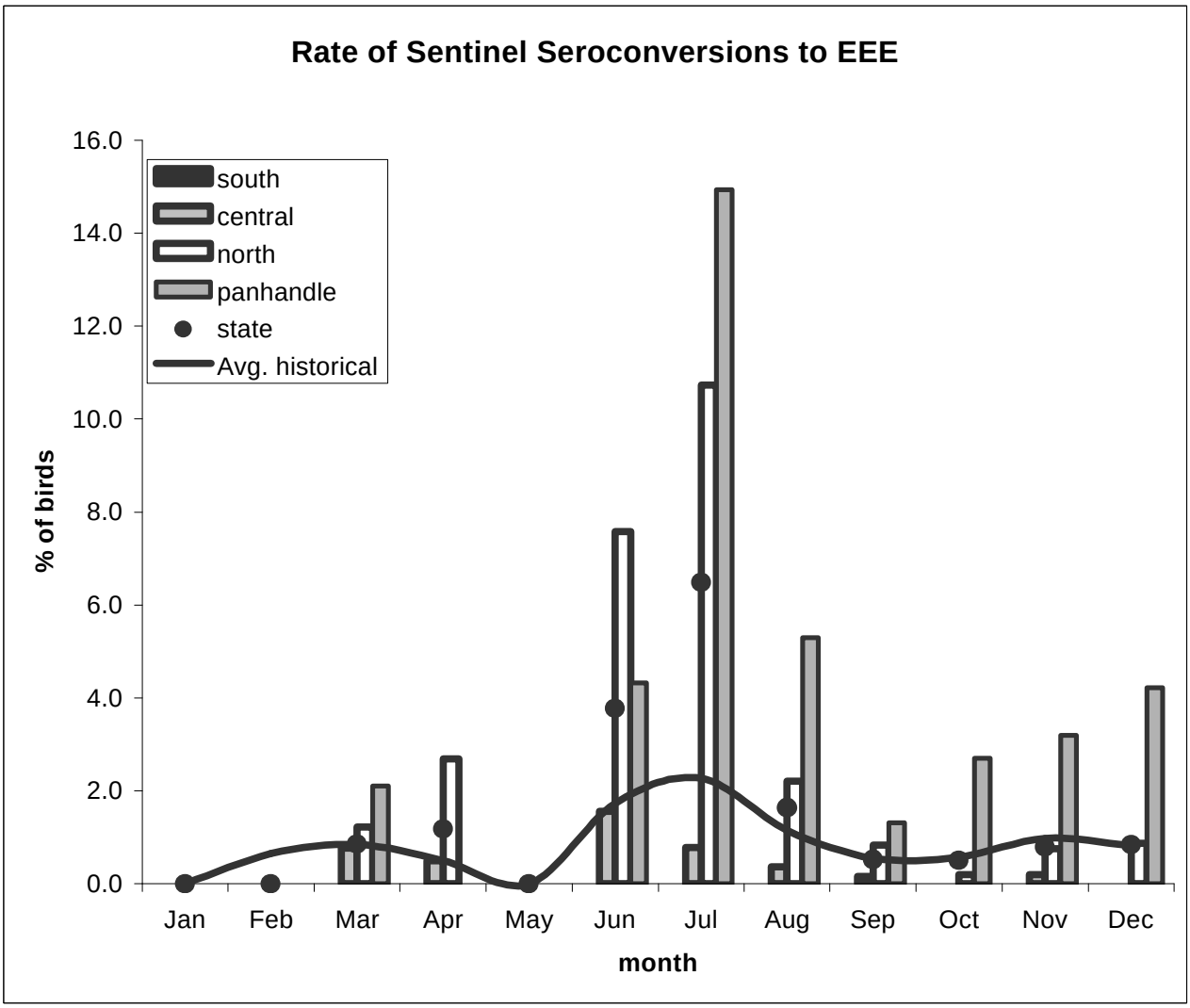

Figure 14. Number of sentinel seroconversions to SLE virus by region by month, 2003
"State" is the statewide rate for that month for 2003; "Avg. historical" is the average statewide rate from 1988 through 2003.

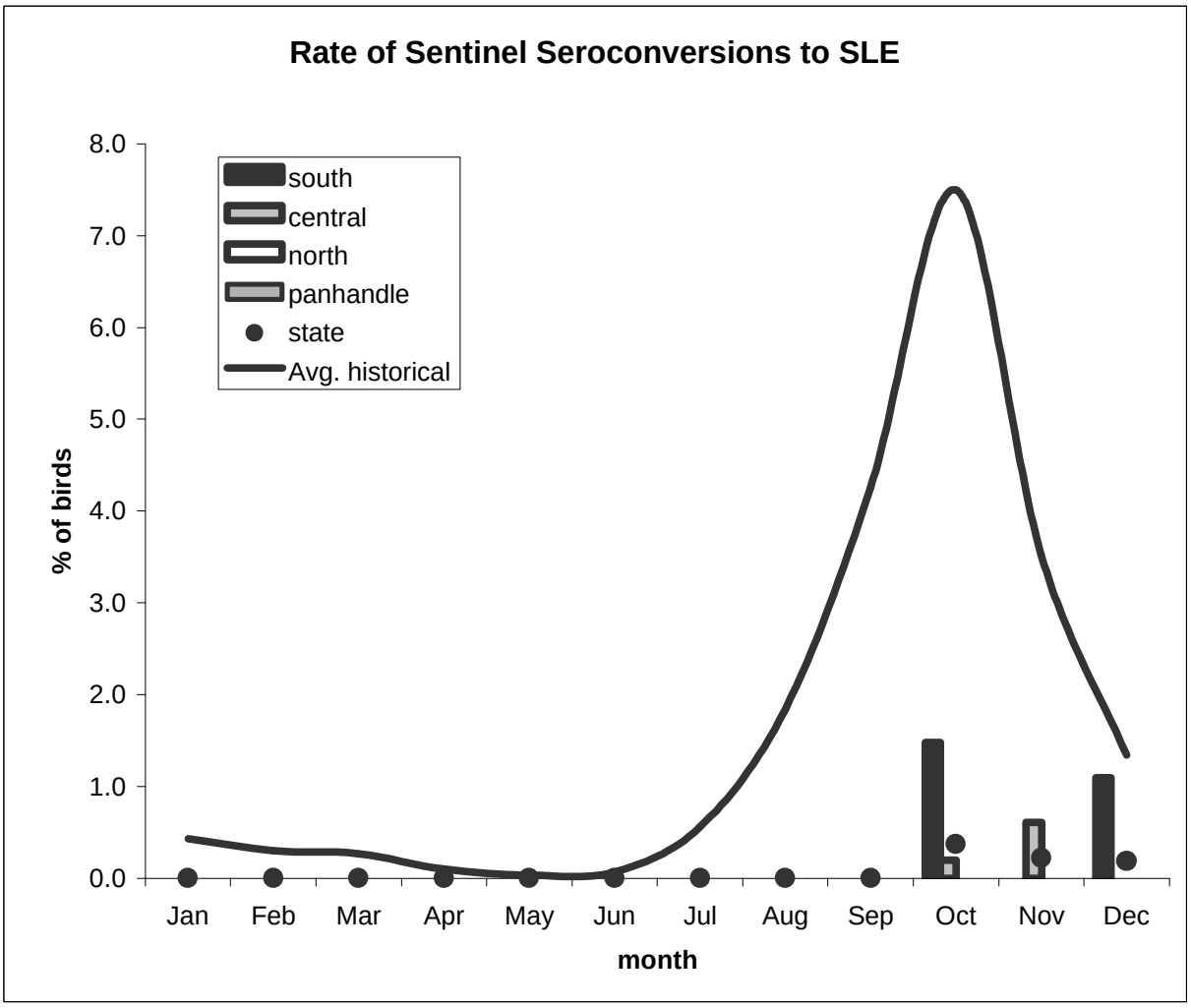

Figure 13. Number of sentinel seroconversions to WN virus by region by month, 2003. ”State” is the statewide rate for that month for 2003; “Avg. historical” is the average statewide rate from 2001 through 2003.