ANTIBODY PREVALENCE OF ST. LOUIS ENCEPHALITIS VIRUS IN AVIAN HOSTS IN LOS ANGELES, CALIFORNIA, 1986

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ABSTRACT. A study was conducted to determine the pattern of St. Louis encephalitis (SLE) virus activity in the avian populations of the Los Angeles metropolitan area in 1986. In total, 679 birds of 42 species were captured at 7 study sites. The overall prevalence of SLE neutralizing (N) antibody of 3% indicated enzootic transmission. Antibody prevalences were higher in birds sampled in the central part of the metropolitan area, which was consistent with other epidemiologic data. The use of specific avian species as sentinels for future surveillance of SLE virus activity was suggested.

INTRODUCTION

Epidemic activity of St. Louis encephalitis (SLE) virus has been reported in California since the 1950s, with cases occurring in rural, agricultural areas, particularly in the San Joaquin Valley. During the last 25 years, only sporadic SLE cases were reported from rural areas in the interior valleys of central California and the southern California deserts. However, a change in this rural pattern of human cases began in 1983 when 3 cases were confirmed in urban-suburban areas of Los Angeles and San Diego counties. The following year, an unprecedented epidemic of SLE occurred in the greater Los Angeles metropolitan area. Between August and October 1984, 26 SLE cases occurred in residents of urban-suburban areas of southern California counties. One additional SLE case was reported in the Los Angeles area in 1985 and three cases in 1986 as well as one case each in suburban Riverside and rural San Bernardino counties in 1985 (Murray et al. 1985; Centers for Disease Control 1985, 1986, 1987).

Historically, the ecology of SLE virus in California was distinct since virus activity occurred predominantly in the irrigated agricultural areas, and transmission cycles involved *Culex tarsalis* Coquillett vector mosquitoes and wild bird species, such as the house finch (*Carpodacus mexicanus*), mourning dove (*Zenaida macroura*), and several species of blackbirds (Reeves and Hammon 1962). Virus activity of SLE in the midwestern and eastern United States appeared as urban outbreaks and involved transmission cycles with *Cx. pipiens* complex mosquitoes and such peridomestic bird species as the house sparrow (*Passer domesticus*) (McLean and Bowen 1980).

Little information was available on the potential avian hosts of SLE in urban settings in southern California; therefore, this study was designed to investigate the pattern of SLE virus activity in the avifauna of the Los Angeles metropolitan area after the continued urban transmission of SLE virus there. The intent was to identify the species of birds that served as the natural hosts for the virus and select specific species and geographic locations for future surveillance.

MATERIALS AND METHODS

Study area. Seven sites were selected in Los Angeles and Orange counties to study the geographic area affected by the previous and current SLE virus transmission to humans and trace possible entries of the virus into the area (Fig. 1). Site 1 was at a tree- and shrub-covered edge between a golf course and small lake in the southern part of the metropolitan area. Sites 2 and 4 were in city parks with scattered large trees and open expanses of maintained grass. Site 3 was a wildlife preserve containing several small lakes surrounded by thick vegetation of shrubs and trees. Sites 2 and 3 were in the central part and site 4 in the northern part of the metropolitan area. Site 5 was an area of second growth of shrubs and small trees next to a dam, golf course, and some agricultural land along the northwestern edge of the city. Site 6 was a riparian habitat along a stream that flowed through a canyon on the northeastern side of the metropolitan area, and site 7 was an abandoned area with some second growth of shrubs and some large trees on a bluff overlooking the Pacific ocean southeast of the Los Angeles area.

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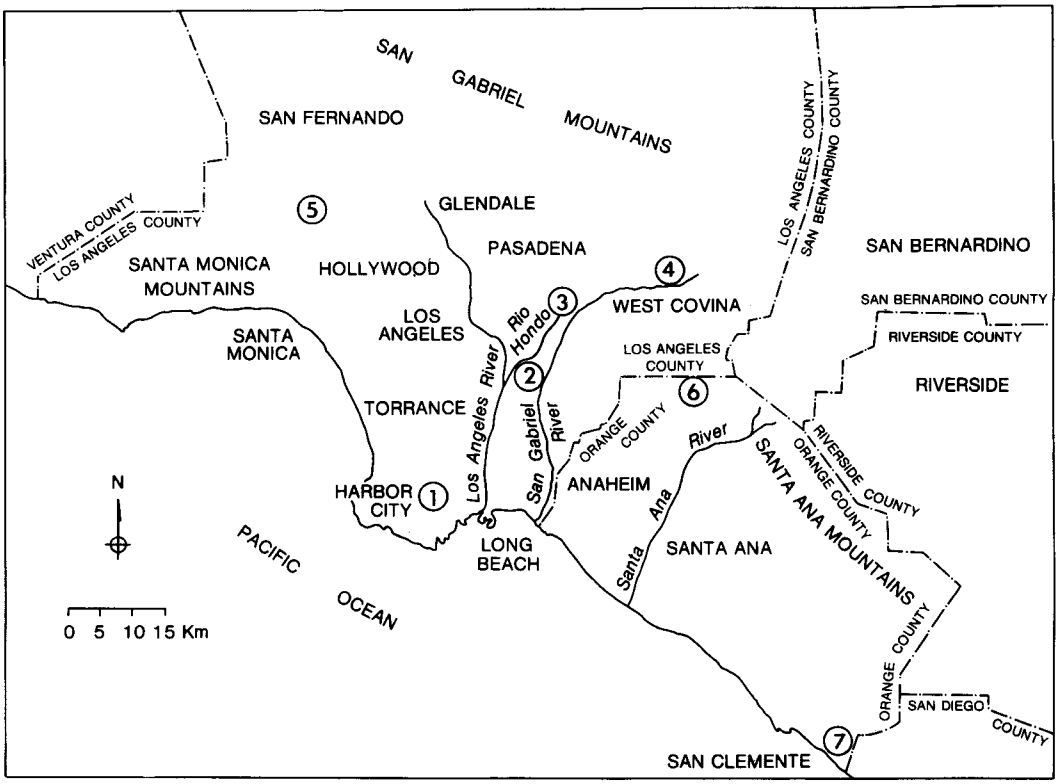

Fig. 1. Map of the greater Los Angeles metropolitan area showing the location of the 7 study sites (circled numbers).

Field procedures. Wild birds were captured with ground-level mist nets placed at sites containing concentrations of such species as the house sparrow, house finch, and blackbirds or in habitats likely to contain desired bird species, such as the song sparrow (*Melospiza melodia*) or northern mockingbird (*Mimus polyglottos*). Casual observations were made on the occurrence and relative abundance of birds at the study sites and in the surrounding areas. Generally, a 0.2 ml sample of blood was taken from the jugular vein of birds using a 1-ml syringe and 26- to 27-gauge needle and was mixed with 0.9 ml of field diluent consisting of medium 199 with antibiotics and 1% bovine albumin and 20% heat-inactivated fetal bovine serum. The blood samples were kept on wet ice until centrifugation and the serum samples were separated and stored in sealed vials at −70°C until tested in the laboratory (Sudia et al. 1967).

Laboratory procedures. Virus isolation. A sample (0.1 ml) of each serum specimen was spread onto monolayer cultures of primary Pekin duck embryo cells (DECC) grown in 6-well plates and was allowed to absorb for 1 hr at 37°C. For standard isolation for a variety of viruses from birds, the cultures were then overlaid with nutrient medium containing 1% Noble agar and 1:25,000 neutral red and incubated at 37°C in 5% CO_2 for up to 10 days. If plaques were observed, the cell cultures were harvested and spread onto additional plates of DECC and the original serum specimen was reinjected. Since no viruses were isolated, the procedures used to identify viruses are not described.

Serology. Heat-inactivated (56°C for 30 min) serum was tested for neutralizing (N) antibody against SLE virus (TBH-28 strain) by the plaque-reduction neutralization test in serially propagated Vero cells grown in 6-well plastic plates (McLean et al. 1983). Equal volumes of serum were mixed with SLE virus diluted to contain approximately 150 plaque-forming units. After incubation overnight at 4°C, 0.1 ml of the mixture was added to the monolayers of Vero cells and allowed to absorb for 45 min. The inoculated cultures were overlaid with agar medium without neutral red and incubated at 37°C in 5% CO_2 for 6 days when they were overlaid again with agar medium containing neutral red.

Cultures were held until plaques were counted; reduction of plaque counts by 80% or more compared with controls was considered positive.

RESULTS

In total, 679 birds of 42 species were captured at the 7 sites in the Los Angeles area (Fig. 1) between August 22–September 6, 1986 (Table 1). The most frequently captured and most widely distributed bird species were the house finch and song sparrow. The house sparrow had a more clumped distribution but was abundant at a few sites. Casual observations of house sparrows and pigeons (*Columbia livia*) indicated they were the predominant bird species within the urban environments, particularly in the central business district. However, no birds were sampled within the central business district and no pigeons were captured during the present study because of the techniques used. The mockingbird was not abundant at any of the sites, with the possible exception of site 3, but was observed throughout the suburban areas. Blackbirds were abundant at a few sites, particularly at Sepulveda Dam (site 5). The mourning dove (*Zenaida macroura*) and spotted dove (*Streptopelia chinensis*) were widely distributed but only moderately abundant; however, these species were difficult to capture with the techniques used and, therefore, only small numbers were sampled.

No virus was recovered from any of the 679 serum samples tested; nevertheless, serologic evidence of SLE virus transmission was obtained (Table 1). The overall prevalence of N antibody (3%) reflected low SLE virus transmission in the metropolitan area; however, the prevalences at individual sites varied greatly. No N antibody was detected in birds at sites 1 and 7, low antibody prevalence (1%) was found at sites 5 and 6, and the highest antibody prevalence was in birds at sites 2–4.

The 2 species of doves had the highest N antibody prevalences (33% and 13%), although there were only 6 and 8 specimens sampled. Thirteen percent of Brewer's blackbirds (*Euphagus cyanocephalus*), all collected at a single site, were positive. Nine percent of the mockingbirds, predominantly from the 3 sites with the greatest evidence of SLE virus activity, had antibody, and 9% of lesser goldfinches (*Carduelis psaltria*) were positive. Except for the song sparrow for which no N antibody positives were detected, the house finch (5%) and house sparrow (3%) had the lowest antibody prevalence of the abundant species.

The overall N antibody prevalence was higher in adult than in immature birds. For 4 species, including the house finch and house sparrow,

Table 1. Neutralizing antibody against St. Louis encephalitis virus in birds captured at various sites in Los Angeles and Orange Counties, California, August 22–September 6, 1986.

Bird species	Harbor Lake (1)	Wilderness Park (2)	Whittier Narrows (3)	Windgate Park (4)	Sepulveda Dam (5)	Tonner Canyon (6)	San Mateo Point (7)	Total
House finch	35	1/5 (20)*	6/36 (17)	5	1/34 (3)	50	2	8/167 (5)
Song sparrow			82	2	11	27	22	144
House sparrow		2/56 (4)	8		10			2/74 (3)
Mockingbird	6	2/9 (22)	1/22 (5)	1/7 (14)	2			4/46 (9)
Brown towhee	8		6	2		26	4	46
Common bushtit	1		4	1	5		11	22
Tri-colored blackbird					18			18
Rufous-sided towhee			4			5	3	12
Yellow-breasted chat							12	12
Lesser goldfinch			1/7 (17)			4		1/11 (9)
Black phoebe	1	1	7			2		11
Black-tailed gnatcatcher							11	11
Yellowthroat	1		6		3		1	11
Loggerhead shrike	1	2	1	4		1	1	10
Brewer's blackbird		1/8 (13)						1/8 (13)
Mourning dove			1/2 (50)		5	1		1/8 (13)
Spotted dove	1	1/4 (25)		1/1 (100)				2/6 (33)
Screech owl						1/2 (50)		1/2 (50)
Other species (24)	3	9	12	7	14	10	4	59
Total	57	7/94 (7)	9/197 (5)	2/29 (7)	1/103 (1)	1/128 (1)	71	20/679 (3)

* Number positive/number tested (% positive).

the prevalence was higher in adults; whereas, for the other 4 species, the prevalence was similar or higher in immature birds (Table 2).

DISCUSSION

The N antibody detected in the birds sampled during this study in 1986 represents, at least in part, current SLE transmission. Of the 20 antibody positive birds, 12 were immature birds exposed no more than a few months before to SLE virus. The positive adult birds could represent an accumulation of infections acquired in a prior season as well as current infections, although this is unlikely because N antibody has been shown to decline with time (McLean et al. 1983). Also natural mortality within the bird population before the study and dilution of the relatively low antibody prevalence that probably occurred during 1985 with seronegative young birds in 1986 would reduce the chances of finding positive adult birds with previously acquired antibody during the current sampling period.

The geographic pattern of the serologic results from the birds captured throughout the Los Angeles metropolitan area during this study was similar to the pattern of SLE virus transmission determined by SLE virus isolations from mosquitoes and the distribution of human cases in 1986. More SLE virus transmission seemed to have occurred within the central and northcentral parts of the metropolitan area. Mosquito isolations were obtained along the San Gabriel River basin near study sites 2 and 3, and human cases occurred near sites 2 and 4 (D. Womeldorf, personal communication). The previous human cases reported during the epidemic in 1984 were more widely dispersed in 4 counties in southern California (Murray et al. 1985). In Los Angeles County in 1984, human cases were reported in the central and southern parts of the county. However, SLE virus isolations from mosquitoes and seroconversions in sentinel chicken flocks were detected only in southern Los Angeles County in the Harbor City area prior to and during the peak in the reported human cases (Murray et al. 1985). Nevertheless, the prevalences of hemagglutination-inhibition (HI) antibody detected in free-ranging birds sampled after the 1984 epidemic reflected the dispersed pattern of SLE transmission in the metropolitan area (Work et al. 1985).

Table 2. Neutralizing antibody against St. Louis encephalitis virus in seropositive species of birds by age in Los Angeles, California, 1986.

Bird species	Immature	Adult	Total
House finch	6/149 (4)*	2/18 (11)	8/167 (5)
House sparrow	0/57	2/17 (12)	2/74 (3)
Mockingbird	3/33 (33)	1/13 (8)	4/46 (9)
Lesser goldfinch	1/10 (10)	0/1	1/11 (9)
Brewer's blackbird	1/8 (13)	0	1/8 (13)
Mourning dove	1/6 (17)	0/2	1/8 (13)
Spotted dove	0/4	2/2 (100)	2/6 (33)
Screech owl	0/1	1/1 (100)	1/2 (50)
Total	12/168 (4)	8/54 (15)	20/322 (6)

* Number positive/number tested (% positive).

The 3% overall prevalence of N antibody in free-ranging birds was low compared to that in other areas that have reported human cases and indicates enzootic transmission. The species composition of the SLE seropositive birds was different from the bird species involved in other urban areas with SLE virus transmission but similar to the species found positive in rural Kern County, California. The house sparrow, mockingbird and mourning dove have also been implicated as SLE hosts in urban SLE epidemics in the midwest and east (McLean and Bowen 1980).

The N antibody prevalence of 3% in house sparrows detected during this study was similar to the 2.1% N antibody and 3.4% HI antibody prevalences found in house sparrows in Memphis, Tennessee, in 1980, when a single human case of SLE was reported in that city (McLean et al. 1983). The mean annual N antibody prevalence for house sparrows and house finches in rural Kern County during 1943–1952 was 6% and 19%, respectively, with an overall mean prevalence of 9% (Reeves and Hammon 1962). The mean overall prevalence of 3% and that for house finches (5%) found in this survey were lower than those found in Kern County. SLE virus transmission in Kern County was predominately rural compared to the suburban-urban type of transmission in the Los Angeles area, although the primary mosquito vector species, *Cx. tarsalis*, may have been the same in both areas (Emmons et al., 1985, Reeves and Hammon 1962).

Since enzootic transmission of SLE virus and a risk of human infection seem to persist in the Los Angeles metropolitan area despite ongoing mosquito control, a second step in the early warning surveillance system to monitor SLE virus activity may be needed. Other metropolitan areas that developed surveillance systems regularly sample the house sparrow populations and rapidly test serum for HI antibody during the transmission season supplemented with monitoring of sentinal chicken flocks established in high risk locations (McLean et al. 1983). However, the low N antibody prevalence found in the house sparrows in the Los Angeles area during this study, their relatively low abundance, and relatively sparse distribution indicates that other species of birds may be more appropriate for monitoring SLE virus activity

in this area. House finches also had a low overall N antibody prevalence but were more abundant and widely distributed than house sparrows. In addition, of the house finches sampled from the 3 sites (2–4) with greatest evidence of virus transmission, 15% were positive. House finches could be used as a sentinel species, but other bird species should be included to insure accurate and sensitive detection of SLE virus transmission. Species of birds in the Columbidae family could also be good sentinel species for SLE surveillance in the Los Angeles metropolitan area since they are abundant, widely distributed, peridomestic, and good hosts for SLE virus (McLean and Bowen 1980). The 2 species of doves sampled in this investigation had high N antibody prevalences and pigeons sampled in 1984 after the SLE epidemic in the Los Angeles area had a high prevalence of HI antibody (Work et al. 1985). Also, pigeons sampled during 8 previous SLE epidemic investigations had a mean antibody prevalence of 40%, and 3 isolations of SLE virus were obtained (McLean and Scott, 1979, McLean and Bowen 1980). Although doves are sometimes difficult to capture in large numbers, pigeons are usually easier to capture with special traps. Several species of doves or pigeons should be included in the sampling scheme to best monitor SLE transmission in the metropolitan area. Pigeons and house sparrows could be captured in the urban sites to supplement the surveillance system and provide monitoring in locations that do not contain many house finches and doves and where captive sentinel birds would not be feasible. The sampling of free-ranging birds (preferably immature birds) for SLE surveillance should be representative over time and geographic area, and test results should be reported quickly to ensure the rapid detection of increased SLE virus activity and increased risk of human infection. Adaptation of the IgM antibody capture enzyme-linked immunosorbent assay for the rapid detection of recent SLE infections in wild passerine birds should greatly benefit local surveillance efforts.

Future testing of the avian populations in the Los Angeles area for SLE antibody could be compared to the specific serologic results on bird species and locations obtained during this study to determine possible seasonal and annual changes in SLE virus activity. Rapid increases in SLE antibody prevalences in target species or rapid geographic spread are indicators of possible increase risk and these conditions should be evaluated in order to determine the need for modifying and/or intensifying mosquito vector control. In Memphis in 1980, the SLE antibody prevalence in free-ranging house sparrows increased from < 1.0% in July to 6–7% in September which alerted the local health department to intensify mosquito control activities (McLean et al. 1983).

ACKNOWLEDGMENTS

The authors thank Mr. Gilbert Challet and Dr. Thomas Monath for their guidance; Drs. Frank Pelsue and Major Dhillon, and Messrs. Dean Harvey and Minoo Madon for their assistance, advice, and support; and Dr. Maurice Ndukwu, Mr. Larry Kirk, Ms. Suzanne Jones, and Ms. Christine Happ for their technical assistance.

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