ST. LOUIS ENCEPHALITIS VIRUS ISOLATED FROM A NESTLING COMMON EGRET IN SOUTHEASTERN MEXICO[1]

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This report describes the isolation of St. Louis encephalitis virus from a nestling Common Egret collected at Minatitlán, State of Veracruz, in September 1965, and presents antibody evidence of infection of resident aquatic birds in southern Mexico.

Introduction

In Mexico, hemagglutination-inhibition (HI) antibodies to group B arboviruses including St. Louis encephalitis (SLE) virus have been reported in sera of humans and domestic and wild mammals (*1-4*); and SLE viral neutralization (N) antibody has been found in sera of humans, domestic animals and herons (*1-5*). Although such antibody tests suggested that SLE virus was widely distributed in Mexico, the virus was not isolated there until 1965 despite tests of thousands of mosquitoes (*6, 7*) and numerous birds (*8*).

Materials and Methods

Study Sites

Virological studies were initiated in the vicinity of Minatitlán in southern Veracruz State during April 1963, when tissues were collected from nestling herons at a colony in freshwater swamp forest near the airport on the north edge of the city. As reported previously, Venezuelan encephalitis, Nepuyo, and Patois group viruses had previously been isolated from habitats near Minatitlán (*9, 10, 11*). The colony, about 2.5 kilometers west of Minatitlán, was one of the principal nesting colonies in southern Veracruz for herons and associated colonial nesting species during 1964-1966. Known as *El Arenal* colony, it was in an area of permanently flooded swamp forest, with trees 20 to 60 feet tall and a dense understory of emergent aquatic vegetation 1 to 6 feet tall.

Even larger nesting colonies were studied on the Pacific coastal lowlands at San Blas—in west central Mexico's State of Nayarit, and at Isla Puntachal in the State of Chiapas, 20 kilometers southwest of Arriaga. Those colonies, respectively containing 2-3,000 and over 5,000 nesting pairs, were both situated in mangrove trees in saline coastal locations. Species sampled at the three colonies are listed in Table 1. Boat-tailed Grackles (*Cassidix mexicanus*) were netted at a night roost in swamps bordering Tampico in Tamaulipas State. These various habitats have been described in more detail and illustrated elsewhere (*12, 13, 14*).

Collection of Blood

Nestling birds were bled by jugular vein puncture, using methods previously described (*8*). Nestlings of large species over a week old were usually returned to their nests, while herons under a week old were usually exsanguinated.

Virus Isolation, Identification, and Antibody Tests

For virus isolation, 10% organ suspensions

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or undiluted red blood cells which had ruptured during freezing and thawing were inoculated intracranially (0.01 ml) and subcutaneously (0.02 ml) into 8 suckling mice. Samples of the inoculated blood and supernatant suspension were then stored at -60°C until negative mouse litters were discarded after 14 days of observation. No other strains of SLE virus were being used in the laboratory during the period when this isolation and reisolation were made.

Hemagglutination inhibition, complement fixation, and plaque reduction neutralization tests in chicken embryonic cell bottle cultures were done as described previously (*15-17*). SLE strain TR9464 (Trinidad) was used in the hemagglutination inhibition (HI) tests. SLE viral neutralization (N) antibodies in selected HI-positive plasmas were detected by virus dilution tests in weanling mice, using the reisolated Mexican strain of SLE 65V310. The Boat-tailed Grackles from Tampico and some nestlings 1-5 days old were only tested for N antibodies. Plasma heated to 60°C for 20 minutes was mixed in equal volume with each of 2 ten-fold dilutions of virus. The mixtures were incubated at 37°C for one hour before intra-cranial inoculation of 0.03 ml per weanling mouse. The mice were then observed for 10-13 days, illnesses and deaths were noted, and $\log_{10}$ neutralization indices (LNI) were calculated. Quantities of virus used were 10-50 weanling mouse intra-cranial 50 per cent lethal doses in four tests, 4 in one test, and 170-500 in three tests. Because excessively high virus challenges were inadvertently used in some tests, negative plasmas could not be detected; thus only positive results (*i.e.*, LNI > 1.5) are reported.

Results

Isolation and Identification of St. Louis Encephalitis Virus

Blood and/or organ pools (heart, lung, and kidney) of 246 nestling herons and associated species (4-20 days of age) collected during 1963-1967 (*8*) from colonies near Minatitlán, Veracruz, were tested by inoculation into suckling mice. One strain of SLE virus was isolated from the blood of a nestling Common Egret (*Egretta alba*–age not recorded), which had been bled on 1 September 1965 at the *El Arenal* colony.

On primary isolation in May 1967 this strain (65V310) killed all eight test mice on the seventh day after intracranial and subcutaneous inoculation; it then continued to kill all inoculated mice in 6, 5, 4-5, and 3 days during four additional passages. The virus was successfully reisolated 13 months later, when blood kept at -60°C killed two of five mice inoculated intracranially and subcutaneously.

A hemagglutinating antigen representing the third passage of the reisolate was not inhibited by polyvalent antisera of the A, C, Bunyamwera, California encephalitis and Guama arbovirus groups; but it was inhibited by a 1:80 dilution of polyvalent group B antiserum and by a 1:320 dilution of SLE antiserum made with the Hubbard strain. (These hemagglutination tests, carried out at pH 6.6, entailed incubation at 37°C for one hour.)

After three passages of the reisolate in suckling mice, a complement-fixing antigen obtained from their brains reacted strongly with two SLE antisera (made by us in rhesus monkeys against strain TR9464 and by the National Center for Disease Control in mice against TBH-28). This antigen reacted only weakly or not at all with the following: Ilheus, Bussuquara, Cowbone Ridge, Rio Bravo, Montana *Myotis* leucoencephalitis and dengue 2 virus hyperimmune mouse ascitic fluids; and Japanese encephalitis guinea pig and Modoc rabbit antisera. Using these same antisera, a plaque reduction virus-dilution neutralization test in chicken embryonic cell cultures yielded LNI > 3.1 with both SLE antisera; LNI 2.2 with Ilheus and Rio Bravo antisera; LNI 2.1 with Bussuquara antiserum; LNI 1.1 with MML antiserum; and LNI 1.0 with Modoc and JE antisera.

Antibodies to St. Louis Encephalitis Virus in Wild Bird Plasmas from Mexico

HI antibodies to SLE virus were found at

TABLE 1–Results of SLE tests for HI and N antibodies in plasmas from nestling herons and associated nesting species collected in southeastern Mexico, 1963-1967.

Common name	Genus and species	Minatitlan, Veracruz		San Blas, Nayarit	Isla Puntachal, Chiapas	
		HI[1]	N[2]	HI[1]	HI[1]	N[2]
Frigate Bird	*Fregata magnificens*				8/46	
Anhinga	*Anhinga anhinga*	4/20	2	0/6		
Olivaceus Cormorant	*Phalacrocorax olivaceus*	3/3	1	0/6	0/2	
Green Heron	*Butorides virescens*	1/1		1/68	0/1	
Common Egret	*Egretta alba*	21/114	12	0/54	1/15	
Snowy Egret	*Egretta thula*	0/2		1/64		
Little Blue Heron	*Egretta coerulescens*			1/60	1/2	
Louisiana Heron	*Egretta tricolor*			0/29	1/22	
Reddish Egret	*Egretta rufescens*			0/1	0/9	
Cattle Egret	*Bubulcus ibis*	0/1			0/3	
Black-crowned Night Heron	*Nyctinassa nycticorax*	0/4			13/38	1
Yellow-crowned Night Heron	*Nyctinassa violacea*			0/10		
Boat-billed Heron	*Cochlearius cochlearius*	7/10	4	2/41		
White Ibis	*Eudocimus albus*				0/1	1

[1]Number with titer $\geq$1:10/number tested.
[2]Number with LNI > 1.5.

low levels (usually between 1:10 and 1:40) in plasmas from 36 of 145 colonial nestling aquatic birds (representing 5 of 8 species) captured at Minatitlán, from 5 of 339 birds (4 of 10 species) captured at San Blas, and from 24 of 139 birds (5 of 10 species) collected at Isla Puntachal. All species with total sample sizes over 10 had some HI-positive birds (Table 1). All nestlings were 3-15 days of age. One four-day old Boat-billed Heron from Minatitlán captured on 2 November 1965 had an HI titer of 10 and a LNI of 2.3. At Isla Puntachal, a Black-crowned Night Heron 3 days old and a White Ibis 2-3 days old had LNI's of > 2.0 and >1.7, respectively; both were captured on 27 August 1967. In addition, two Boat-tailed Grackles about 15 months of age, collected at Tampico in August 1969, had SLE viral neutralization (N) antibodies (LNI 2.6 and 3.2).

Most birds with N antibodies also had HI antibodies. For example, six of seven Boat-billed Herons from Minatitlán with an HI titer of 10 were N-tested; four were positive (LNI >1.6), one equivocal (LNI 1.2) and one negative (LNI > 1.0). In contrast, the single Green Heron from San Blas that was positive for HI antibodies was negative for N antibodies.

Discussion

The extensiveness of cross-reactions between members of the group B arboviruses in serological tests is legend; thus serological evidence establishing the presence of SLE or other group B arboviruses in Mexico (*1-4*) had been inconclusive. However, the isolation of SLE virus just described establishes the existence of this virus in Mexico.

Birds are generally considered to constitute the principal group of vertebrate amplifying hosts for SLE virus in nature (*18, 19*). Many of the positive results of antibody tests of avian plasmas presented herein indicated presence of maternal antibodies in nestling herons because of the low HI titers found together with positive LNI's in young birds 2-15 days old. In contrast, the N antibody found in Boat-tailed Grackles over one year of age represented previous infections. These antibody data are thus compatible with the involvement of birds during 1963-1969 in the amplification of a group B arbovirus, presumably SLE, along Mexico's tropical Atlantic and Pacific coasts.

Summary

One strain of St. Louis encephalitis virus

(65V310) was isolated from the blood of a nestling Common Egret (*Egretta alba*) from a large nesting colony of mixed species of herons located near Minatitlán, Veracruz, on Mexico's gulf coast lowlands. This was the first isolate from Mexico of SLE virus, an important group B arbovirus pathogenic to man.

Hemagglutination inhibition antibodies to group B arboviruses, probably SLE, were found in plasmas from 36 of 145 young of colonial nesting aquatic birds caught near Minatitlán, 5 of 339 from San Blas in the State of Nayarit, and 24 of 139 from Isla Puntachal on the Pacific lowlands of southern Mexico. SLE viral neutralization antibodies were found to the Mexican SLE isolate in 23 individuals of seven aquatic-associated bird species. These data indicate the probable widespread distribution of this virus and the involvement of birds during 1963-1969 in the amplification of group B arboviruses, presumably SLE virus, in Mexico.□

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NEW UNITED STATES IMPORT RESTRICTIONS ON PSITTACINE BIRDS

Effective 10 March 1972 the United States Department of Agriculture (USDA) promulgated regulations placing new import restrictions on psittacine birds (parrots, macaws, and other birds of the Order *Psittaciformes*). the New USDA regulations are aimed primarily at preventing introduction of exotic strains of Newcastle Disease. They superimpose additional requirements on existing Public Health Service regulations concerning importation of these birds intended to guard against psittacosis.

The USDA requires all entering or returning psittacine birds, including personal pets, to undergo isolation and approved medication for 45 days at a facility located overseas and approved by the USDA. It also requires a 30-day post-entry isolation period in the United States at approved facilities. Ports of entry for incoming birds are now restricted to Honolulu, Los Angeles, Miami, New York, and Seattle. Birds from Mexico may also enter at San Isidro, California, and those from Canada may enter at Buffalo, New York or Detroit, Michigan. [*Weekly Epidemiological Record* of the World Health Organization 47(16)160, 1972.]