ST. LOUIS ENCEPHALITIS VIRUS TRANSMISSION TO EMUS (DROMAIUS NOVAEHOLLANDIAE) IN PALM BEACH COUNTY, FLORIDA WITH EVIDENCE OF WESTERN EQUINE ENCEPHALITIS VIRUS ANTIBODY TRANSPORT TO FLORIDA BY EMUS INFECTED IN OTHER STATES

JONATHAN F. DAY¹ AND LILLIAN M. STARK²

¹Florida Medical Entomology Laboratory, Institute of Food and Agricultural Sciences
University of Florida, 200 9th St. SE, Vero Beach, FL 32962
²Tampa Branch Laboratory-Virology Section
Florida Department of Health and Rehabilitative Services
3952 West M. L. King, J r. Boulevard, Tampa, FL 33614

ABSTRACT

From November 1993 through January 1995, sera were collected from 59 domestic emus (Dromaius novaehollandiae) at a ranch in Palm Beach County, FL and tested for antibody evidence of arboviral infection. Hemagglutination inhibition (HI) and neutralizing (NT) antibodies to St. Louis encephalitis (SLE) virus were identified in sera collected each year. In addition, HI and NT antibodies to eastern equine encephalitis (EEE) virus and NT antibodies to western equine encephalitis (WEE) virus were detected in emus imported to and then maintained at the ranch. Neither of the equine
encephalitis viruses are common in Palm Beach County and many of the EEE and WEE antibody-positive emus were imported from other states prior to vaccination. Emus imported from California, Louisiana and Texas had evidence of naturally acquired antibodies to EEE and WEE viruses. This observation underscores the potential threat of arboviral introduction by infected vertebrates and emphasizes the importance of instituting quarantine procedures to regulate the transport of hosts that may be infected with arboviral agents.

Key Words: Encephalitis virus, SLE, EEE, WEE, emu

RESUMEN

Desde noviembre 1993 hasta enero 1995 se colectaron sueros de 59 emús domésticos (Dromaius novaehollandiae) ubicados en una finca en el condado de Palm Beach, Florida, y se examinaron para determinar la presencia de anticuerpos contra infección arboviral. La inhibición de la hemoaglutinación (HI) y los anticuerpos neutralizadores (NT) del virus de la encefalitis de St. Louis (SLE) fueron identificados en los sueros colectados anualmente. Además, la HI y el NT del virus de la encefalitis equina oriental (EEE) y el NT del virus de la encefalitis equina occidental (WEE) fueron detectados en emús importados a la finca y luego mantenidos allí. Ninguno de los dos virus de encefalitis equina son comunes en el condado de Palm Beach y muchos de los emús que resultaron tener anticuerpos contra EEE y WEE habían sido importados de otros estados antes de recibir vacunaciones. Los emús importados de California, Louisiana, y Texas tenían evidencia de anticuerpos para EEE y WEE adquiridos naturalmente. Esta observación enfatiza la amenaza potencial de la introducción de arbovirus en vertebrados infectados y subraya la importancia de establecer procedimientos de cuarentena para reglamentar el transporte de hospederos infectados con arbovirus.

Naturally occurring mosquito-borne arboviruses infect and cause mortality in domestic emus (Dromaius novaehollandiae Linn.). In Texas, emus from 8 flocks were infected with western equine encephalitis (WEE) virus in 1992 and suffered 15-50% morbidity and 8.8% mortality (Ayers et al. 1994). In 1991, emus were infected with eastern equine encephalitis (EEE) virus in Louisiana, where an 87% mortality rate was reported (Tully et al. 1992). Two emus died of EEE virus infection in Georgia during the summer of 1992 (Brown et al. 1993). In Volusia County, FL during the spring of 1992, EEE-related morbidity and mortality rates in an emu flock were 40.5% and 14.1%, respectively. This outbreak resulted in a one year financial loss of an estimated $192,000 (Day and Stark 1996a).

In southern Florida and many parts of the central USA, St. Louis encephalitis (SLE) virus is the most commonly transmitted mosquito-borne arbovirus (Chamberlain 1980, Day & Stark 1996b). However, SLE infection in emus maintained in Florida, and in other areas of North America where SLE is endemic, has been reported only once (Day & Stark 1996b).

Eastern equine encephalitis virus, as well as antibody evidence of its transmission, is reported frequently in the northern half of Florida, but is rarely found in the 10 counties comprising the southern tip of the state (Day & Stark 1996b). The WEE virus is found west of the Mississippi drainage basin (Reisen & Monath 1989). However, WEE virus comprises a complex of at least six serologically related but distinct viruses. One of these, Highlands J (HJ) virus, is present in central and north Florida, but is absent from the southern tip of the state (Trent & Grant 1980).
The purpose of our study was to monitor natural arboviral transmission to emus in Palm Beach County, FL. Additionally, we present evidence that emus imported into Florida were initially infected with EEE and WEE viruses in the central and western USA.

**Materials and Methods**

**Study Site**

Emus were maintained at a 4 ha ranch in Palm Beach County (26°40'N, 80°15'W), FL. The ranch was located within a slash pine (Pinus elliottii Engelm.)/saw palmetto (Serenoa repens (Bartr.) Small] habitat in the western part of the county.

During the spring of 1993, approximately 150 juvenile emus were imported to the ranch from locations in Florida, California, Louisiana, Pennsylvania and Texas. Our study began in November 1993 in response to emu morbidity that was suspected of being caused by an arboviral agent. The study continued through January 1995.

When the first blood sample was taken, emus were sexed and placed into one of 3 age groups: hatching year (HY) = 1-120 days old, juvenile (J) = 121-365 days old, and adult (A) = ≥366 days old.

**Serum Collection and Analysis**

Blood was drawn from the jugular veins of restrained emus. A 3.0 ml sample was collected from HY emus, and a 5.0 ml sample from J and A birds. Blood samples were allowed to clot overnight at room temperature, were centrifuged at 3,400 x g for 30 min, and the resulting sera used for SLE and EEE virus hemagglutination inhibition (HI) and neutralizing (NT) antibody assays. All sera were analyzed for HI antibody to SLE and EEE viruses and for NT antibody to SLE virus. Selected sera were also analyzed for NT antibody to EEE and WEE viruses.

A micro-adaption of the HI antibody test of Beaty et al. (1989) was used with a hemagglutinin (HA) prepared from a Florida human SLE isolate (TBH-28, Florida Department of Health and Rehabilitative Services, Tampa Branch Laboratory). Additionally, all sera were examined in the same manner for HI antibody against EEE virus using an HA prepared from a Florida human isolate (NJ-60, Florida Department of Health and Rehabilitative Services, Tampa Branch Laboratory). The methodology used for HI antibody testing is described in detail by Day et al. (1996).

Aliquots of all sera were examined for NT antibody to SLE virus by serial virus dilution with undiluted serum (Beaty et al. 1989). The challenge virus was a Florida isolate (SLE-P15, Florida Department of Health and Rehabilitative Services, Tampa Branch Laboratory) obtained from a pool of Culex nigripalpus Theobald mosquitoes. Two NT tests for EEE antibody were performed on selected serum aliquots. The challenge viruses for these tests were a 1964 Florida human isolate (D64-837, Florida Department of Health and Rehabilitative Services, Tampa Branch Laboratory) and an isolate (VO-73, Florida Department of Health and Rehabilitative Services, Tampa Branch Laboratory) from a pool of 7 Cx. erraticus (Dyar and Knab) collected in August 1992 in Volusia County, FL (Day & Stark 1996a). A single NT test for WEE antibody was performed on selected serum aliquots. The challenge virus for these tests was WEE Fleming strain (VR-1251, American Type Culture Collection, Rockville, MD). We tested for cross reactivity of the VR-1251 strain with Florida EEE and Hj viral strains and found none.

The LD_{50} virus dilutions for each series of serum-virus mixtures, along with that of the control, were determined to a single decimal point. A logarithmic LD_{50} was ex-
pressed as the exponent of the reciprocal of the endpoint dilution. The log neutralization index of each serum was obtained by subtracting its $LD_{50}$ from that of the control. Indices of $<1.0$ were considered negative, $1.0$ to $1.6$ were equivocal and those $\geq 1.7$ were positive. The methodology used for NT antibody testing is described in detail by Day et al. (1996).

Viral Isolation Attempts

Blood from 10 emus and tissue (brain, heart, liver and spleen) from 2 emus that died of encephalitis-like symptoms were assayed for arboviral agents as described below. One drop of blood was mixed in 0.7 ml of laboratory-prepared biological field diluent (BFD) (90% Minimum Essential Medium with Hank’s salts (Sigma Chemical Co., St. Louis, MO), 10% fetal bovine serum (Intergen Co., Purchase, NY), 200 U/ml penicillin (Sigma Chemical), 200 ug/ml streptomycin (Sigma Chemical), 2.5 ug/ml amphotericin B (Sigma Chemical), and 50 ug/ml kanamycin (Sigma Chemical). Blood samples were placed immediately on wet ice in the field and transported to the laboratory where they were stored at -70°C until analysis.

Approximately 0.5 g of tissue was pulverized in 0.7 ml BFD with a chilled 1.0 ml Potter-Elvehjem tissue grinder (Fisher Scientific, Orlando, FL) in a laminar flow biosafety cabinet. The suspension was clarified by centrifugation at $800 \times g$ and rendered free of bacteria either by centrifugation at $4,300 \times g$ or filtration with a 0.2 um syringe filter. The supernatant was aliquoted into sterile 1 ml polypropylene cryopreservation vials (Fisher Scientific, Orlando, FL) and stored at -70°C until analysis as described by Day et al. (1996).

Vaccination and Maternal Antibody Transfer

During April and May 1994, selected emus were vaccinated with 1.0 ml of Encephaloid IM®, an inactivated EEE/WEE vaccine (Ft. Dodge Laboratories, Ft. Dodge, IA). Emus were bled before vaccination to establish baseline titers and periodically following vaccination to track resulting antibody titers. Chicks from hens with natural SLE infections were bled within 24 days of hatching to determine the presence or absence of maternally acquired SLE antibody.

Statistical Tests

Statistical differences in EEE antibody titers (HI and NT) before and after vaccination were tested by using unplanned tests of the homogeneity of replicates tested for goodness of fit (G-statistic) (Sokal and Rohlf 1981).

**RESULTS**

Sera from 59 emus maintained at the Palm Beach County ranch were tested between November 1993 and January 1995 for evidence of arboviral transmission. Twenty-six of the emus originated in California, 13 in Florida, 11 in Louisiana, 8 in Texas and one in Pennsylvania. Twenty-four of the emus were bled more than once. Five were HY, 34 were J and 20 were A age group when first bled.

Most of the emus had HI (43 of 59, 73%) and NT (44 of 59, 75%) antibody to SLE virus. Antibody titers for individual emus were as follows. SLE HI antibody titers: 1:10 = 2 emus, 1:20 = 5, 1:40 = 2, 1:80 = 6, 1:160 = 15, 1:320 = 11 and $640 = 2$. SLE NT antibody titers: $1.8 = 6$ emus, $2.0 = 3, 2.1 = 4, 2.3 = 5, 2.4 = 1, 2.6 = 1, 2.7$
Twenty-five percent (15 of 59) had HI antibody to EEE virus, 32% (16/50) had NT antibody to EEE virus, and 57% (8 of 14) had NT antibody to WEE virus (Fig. 1). Antibody titers (HI and NT for EEE and WEE viruses) for individual emus appear in Table 1.

No virus isolations were made from the blood of 10 emus that displayed encephalitis-like symptoms nor from the tissues of 2 emus that died of encephalitis-like symptoms in 1993.

Sera from 5 emus were tested for antibody titers prior to EEE/WEE vaccination. Four of 5 had detectable HI and NT titers to SLE virus resulting from natural infections. None of the birds had detectable HI titers, but one had positive NT titers to EEE virus. Eight emus (the 5 described above plus 3 additional birds) were tested for antibody titers following vaccination. Six of 8 had HI and NT antibody titers to naturally acquired SLE virus. The mean age of vaccinated birds was 408 ± 37 (SD) days (range = 362-464 days). The mean number of days between vaccination and the first serum sample was 27 ± 14 days (range = 22-52 days). There was a significant increase in the proportion of emus with HI antibody titers (P < 0.05, G = 8.95, df = 1) and NT antibody titers (P < 0.05, G = 8.55, df = 1) to EEE virus following vaccination.

Fig. 1. Arboviral antibody seropositive rates for emus maintained at the West Palm Beach ranch during 1993 and 1994. Abbreviations are as follows: SLE = St. Louis encephalitis, EEE = eastern equine encephalitis, WEE = western equine encephalitis, HI = hemagglutination inhibition antibody and NT = neutralizing antibody.
**TABLE 1. PROBABLE SOURCE OF HI AND NT ANTIBODY TO EEE AND WEE VIRUSES IN EMUS MAINTAINED AT A RANCH IN PALM BEACH COUNTY, FLORIDA: 1993-94.**

<table>
<thead>
<tr>
<th>Point of emu origin</th>
<th>Number imported to Florida</th>
<th>Number tested for HI antibody</th>
<th>Number tested for NT antibody</th>
<th>Source of antibody &amp;ntimes;</th>
<th>No. EEE-positive/no. tested</th>
<th>NT positive/no. tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>California</td>
<td>26</td>
<td>26</td>
<td>23</td>
<td>Nat.</td>
<td>0/26</td>
<td>0/23</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mat./Vac.</td>
<td>7/26</td>
<td>4/8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>26</td>
<td>0/8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>21</td>
<td>1/8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4/8</td>
<td>1 = &gt;4.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2 = 3.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 = 2.5</td>
<td></td>
</tr>
<tr>
<td>Louisiana</td>
<td>11</td>
<td>11</td>
<td>8</td>
<td>Nat.</td>
<td>1/11</td>
<td>2/4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mat./Vac.</td>
<td>3/11</td>
<td>2/4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5/8</td>
<td>1 = &gt;1.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2 = 1.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3 = 2.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 = &gt;2.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2/4</td>
<td>both = 2.5</td>
</tr>
<tr>
<td>Texas</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>Nat.</td>
<td>0/8</td>
<td>1/2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mat./Vac.</td>
<td>0/8</td>
<td>0/2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0/8</td>
<td>0/2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0/2</td>
<td>= 2.7</td>
</tr>
<tr>
<td>Totals:</td>
<td>45</td>
<td>45</td>
<td>39</td>
<td>Mat./Vac.</td>
<td>7/45</td>
<td>0/14</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5/39</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0/14</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1/14</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1/14</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7/14</td>
<td></td>
</tr>
</tbody>
</table>

1Mat./Vac. = maternal antibody transfer by a naturally infected or vaccinated hen, Nat. = natural infection, Vac. = vaccinated emu.
Natural EEE transmission is rare in Palm Beach County (Day & Stark 1996b). Because many of the emus in our study were HI and NT antibody-positive for EEE virus and because many of the birds originated west of the Mississippi River, selected sera were tested for NT antibody to WEE virus. Most of the sera collected before the emus were treated with an EEE/WEE vaccine. Therefore, positive findings in these birds indicated the possibility of natural infection or maternal antibody transfer (Day & Stark 1996a). In Volusia County, Florida, we observed that emus naturally infected with EEE virus usually had NT antibody titers >2.0. Newly hatched emus with maternally derived antibody titers (through natural infection or vaccination of the hens) usually displayed NT antibody titers <2.0. Eight of 14 emus tested for NT antibody to WEE virus were positive, all but one with NT antibody titers >2.0, indicating possible natural infection at their hatching site. One of 45 (2%) HI tests and 4 of 39 (10%) NT tests indicated EEE antibody titers that most likely resulted from natural infection (Table 1).

Four of 5 newly hatched chicks from hens with a natural SLE infection had HI and NT antibody titers to SLE virus. The mean age of the chicks was 11.2 ± 8.1 days (range = 6-24 days) when they were first tested for antibody. All had negative EEE antibody titers.

**DISCUSSION**

The introduction of exotic hosts into habitats that have active arbovirus transmission provides a potential vehicle by which transfer of virus from one location to another may be facilitated. Emus are susceptible to infection by EEE virus (Tully et al. 1992, Brown et al. 1993, Day & Stark 1996a), WEE virus (Ayers et al. 1994) and SLE virus. An important, and yet unanswered question is whether and for how long infected emus circulate virus titers sufficient to infect mosquitoes or other potential vectors. Emus are sold and transported throughout North America and can potentially transport an arbovirus from an active focus to an area where the virus is not extant but vectors suitable to establish an active viral focus are present.

The consistently high SLE antibody levels in emus from Palm Beach County indicate that these birds were most likely infected naturally with SLE virus during the autumn of 1993 when an unusually high level of SLE transmission was reported in sentinel chickens along the east-central coast of Florida (Stark, unpublished data). The emu ranch was located in a habitat favored by the major vector of SLE virus in Florida, *Cx. nigripalpus*, and it is likely that an SLE transmission focus involving mosquitoes, emus and wild birds was established around the emu ranch during 1993. It is also possible that some of the emus were infected with SLE virus in California, Louisiana or Texas prior to their import into Florida. However, the facts that the majority of emus in the flock were HI and NT antibody positive (73% and 75% respectively) for SLE virus and that the titers for both types of antibody were high, indicates that SLE transmission was not sporadic and, regardless of the state of origin of the emus, virtually all of them ended up SLE positive at the West Palm ranch.

No viral isolates were made from sick or dead emus in 1993. Judging from the high SLE antibody rates and the low morbidity and mortality rates among infected birds, it does not appear that SLE virus causes as severe an infection nor as high a mortality rate as do EEE and WEE viruses.

Natural transmission of EEE and HJ viruses is uncommon in Palm Beach County (Day & Stark 1996b). However, 15 of 59 (25%) of the emus in our study had HI antibody titers and 16 of 50 (32%) had NT antibody titers to EEE virus. Additionally, 8 of 14 (57%) had NT antibody titers to WEE virus. Forty-six (78%) of the emus in our
day and stark: sle transmission to florida emus

study originated outside of florida (one emu, not shown in table 1, originated in pennsylvania). five of the 13 emus that originated in florida hatched at the palm beach facility, whereas 8 were imported from a ranch in volusia county where eee virus is endemic (day & stark 1996a). some of the antibody-positive emus reacted to eee or wee tests because of vaccination or maternally-derived antibody. however, judging from the high antibody titers, at least some of the emus had antibody acquired as the result of natural infection at their hatching location (table 1). emus and other exotic avian hosts may potentially transport live virus across state lines. it is important to determine the extent and duration of viremias in infected emus to evaluate the risk of viral introduction by emus infected at one site and then transported to a secondary or even a tertiary location supporting suitable vector populations.

acknowledgments

william kohl, nazar hussain, arnie croteau and tersa lowry assisted with this study. this research was funded by a research contract from the florida emu association. florida agricultural sciences experiment station journal series no. r-05160.

references cited

ayers, j. r., t. l. Lester, and a. b. angulo. 1994. an epizootic attributable to western equine encephalitis virus infection in emus in texas. j. am. vet. med. assoc. 205: 600-601.

beaty, b. j., c. h. calisher, and r. e. shope. 1989. arboviruses, pp. 797-855 in: schmidt, n. j. and r. w. emmons [eds.], diagnostic procedures for rickettsial and chlamydial infections. am. public health assoc., washington, dc.


chamberlain, r. w. 1980. history of st. louis encephalitis. chapter 1 in monath, t. p. [ed.], st. louis encephalitis. am. public health assoc., washington, dc.

day, j. f., and l. m. stark. 1996a. eastern equine encephalitis transmission to emus (dromaius novaehollandiae) in volusia county, florida: 1992 through 1994. j. am. mosq. control assoc. 12: 429-436.


day, j. f., l. m. stark, j.-t. zhang, a. m. ramsey, and t. w. scott. 1996. antibodies to arthropod-borne encephalitis viruses in small mammals from southern florida. j. wildlife dis. 32: 431-436.

reed, l. j., and h. a. muench. 1938. a simple method of estimating fifty percent endpoints. am. j. hyg. 27: 493-497.


sokal, r. r., and f. j. rohlff. 1981. biometry, 2nd ed. w. h.freeman and co., san francisco, ca.

trent, d. w., and j. a. grant. 1980. a comparison of new world alphaviruses in the western equine encephalitis virus complex by immunochemical and oligonucleotide fingerprint techniques. j. gen. vir. 47: 261-282.