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EASTERN EQUINE ENCEPHALITIS TRANSMISSION TO EMUS (*DROMAIUS NOVAEHOLLANDIAE*) IN VOLUSIA COUNTY, FLORIDA: 1992 THROUGH 1994[1]

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ABSTRACT. From May 1992 through October 1994, sera were collected from 204 domestic emus (*Dromaius novaehollandiae*) at a ranch in Volusia County, FL, and tested for antibody evidence of arboviral infection. Hemagglutination-inhibition (HI) and neutralizing (NT) antibodies to eastern equine encephalitis (EEE) virus were identified in sera collected during each year. In addition, HI and NT antibodies to St. Louis encephalitis virus were detected in 3 naturally infected emus. Isolations of EEE virus were made from emu blood and tissues collected in 1992 and 1994, when EEE-related mortality in emus was 14% and 1%, respectively. A total of 259 mosquito pools was collected and tested for arboviruses during the 3-year study. The EEE virus was isolated from 4 of 140 mosquito pools (2 *Anopheles crucians* and 2 *Culex erraticus* pools) in 1992 and 3 of 10 pools (all *Culex nigripalpus*) in 1994. Emus vaccinated against EEE virus showed evidence of short-term HI antibody acquisition. Evidence of EEE antibody transfer from naturally infected hens to their offspring is reported.

INTRODUCTION

The introduction of emus (*Dromaius novaehollandiae* Linn.) to North America from Australia as part of a speculative commodity market has resulted in the establishment of emu ranches throughout the continent. These ranches are particularly abundant in southern states including Texas, Louisiana, Georgia, and Florida. Maintenance of emus in southern climates places individuals of this exotic avian species in contact with arthropods infected with arboviral agents including, but not limited to, St. Louis encephalitis (SLE) virus, eastern equine encephalitis (EEE) virus, western equine encephalitis (WEE) virus, Venezuelan equine encephalitis virus, and Highlands J virus. There is evidence that emus are susceptible to, and adversely affected by, WEE (Ayers et al. 1994) and EEE (Tully et al. 1992, Brown et al. 1993) viruses. If emus serve as efficient amplification hosts for mosquito-borne encephalitis viruses, then the widespread introduction of these hosts into endemic areas may create a significant health threat for humans and domestic animals, resulting in unanticipated vector control problems. Presently, no EEE or WEE vaccine is marketed for emus; however, commercially available horse vaccines are often adapted for use in these birds.

Maternally derived arboviral antibody is transferred from naturally infected or vaccinated horses to their offspring (Gibbs et al. 1988). In addition, maternal antibody transfer occurs in wild birds naturally infected with SLE and WEE viruses (Sooter et al. 1954) and in domestic birds infected with arboviruses (Reeves et al. 1954, Bond et al. 1965).

The purpose of our study was to monitor natural arboviral transmission to emus maintained in Volusia County, FL. In addition, we evaluated the development of antibody titers in vaccinated emus and maternal antibody transfer from EEE-infected hens to their chicks.

MATERIALS AND METHODS

Study site: Emus for this study were maintained at an 8.1-ha ranch in Volusia County (29°01′N, 81°05′W), FL. The ranch originally served as a chicken farm and was located adjacent to a bald cypress (*Taxodium distichum* (Linn.))/southern red maple (*Acer rubrum* Linn. var. *tridens* Wood) marsh. In August 1991, 108 juvenile emus were brought to the ranch. Our study began in May 1992, in response to emu mortality that was suspected of being caused by an arboviral agent, and continued through October 1994.

Emus were sexed and placed into one of 3 age groups: hatching (H) = 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 H emus, and a 5.0-ml sample from J and A birds. Blood samples were allowed to clot overnight at room temperature then were centrifuged at 3,400 × *g* for 30 min and the resulting sera used for SLE and EEE virus hemagglutination-inhibition (HI) and neutralizing (NT) antibody assays. A total

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of 322 sera was tested for HI antibody to EEE and SLE viruses during the 3 study years.

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

Aliquots of most sera were examined for NT antibody by serial virus dilution with undiluted serum (Beaty et al. 1989). The challenge virus for SLE was a Florida isolate (SLE-P15) obtained from a pool of *Culex nigripalpus* Theobald mosquitoes. Two NT tests for EEE antibody were performed on serum aliquots. The challenge viruses for these tests were a 1964 Florida isolate from a human (D64-837), and an isolate (VO-73) from a pool of 7 *Culex erraticus* (Dyar and Knab) collected in August 1992 at the Volusia County emu ranch. Three hundred and thirty-two sera were tested for NT antibody to EEE virus during the 3 study years. Sera collected from 97 emus in 1994 were tested for NT antibody to SLE virus. The methodology used for NT antibody testing is described in detail by Day et al. (1996).

In 1992, 22 (7 J and 15 A) emus were tested. Four were bled twice and one was bled 3 times, resulting in a total of 27 serum samples. Sera from 19 emus were analyzed for HI antibody. Sera from 6 emus were analyzed for NT antibody to EEE virus. Blood from 17, sera from 3, fecal samples from 8 and tissue samples from 7 emus were analyzed for viral agents.

In 1993, 64 (53 H, 7 J, and 4 A) emus were tested. Of these, 17 were bled once, 27 twice, 11 three times, 8 four times, and one 6 times. Beginning in 1993, all sera were routinely separated into 2 aliquots; one for HI antibody analysis to SLE and EEE viruses and the second for NT antibody analysis to EEE virus. No viral isolation attempts were made during 1993.

In 1994, 118 (72 H and 46 J) emus were tested. Of these, 76 were bled once, 21 twice, 12 three times, 8 four times and one 5 times. Serum from each emu was analyzed for HI antibody to SLE and EEE virus and NT antibody to EEE virus. Sera from 97 emus were analyzed for NT antibody to SLE virus. Blood and/or tissue samples from 16 emus were tested for virus.

Vaccination and maternal antibody transfer: Beginning in May 1993, selected emus were vaccinated with 0.2 (H emus), 0.5 (J emus), or 1.0 ml (A emus) of Encephaloid IM®, an inactivated EEE/WEE vaccine (Ft. Dodge Laboratories, Ft. Dodge, IA). Emus were vaccinated up to 3 times at 1-month intervals. They were bled before vaccination to establish baseline titers and periodically following vaccination to track resulting antibody titers.

Chicks from hens that received a known EEE virus challenge were bled immediately after hatching, and periodically thereafter, to determine the level and duration of maternally acquired antibody. Hens were divided into one of 4 EEE virus challenge categories: (− −) = hens without EEE antibody and with no known exposure to EEE virus or vaccine, (+) = vaccinated hens with no known natural exposure to EEE virus; (++) = hens that suffered a mild natural EEE infection, (+++) = hens that suffered a severe, near fatal natural EEE infection.

Virus isolation attempts: Blood for virus isolation was collected from emus as described above. 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 μg/ml streptomycin [Sigma Chemical], 2.5 μg/ml amphotericin B [Sigma Chemical], and 50 μg/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 (brain, heart, liver, spleen, or intestine) from emus that died of encephalitis-like symptoms 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 × *g* and rendered free of bacteria either by centrifugation at 4,300 × *g* or filtration with a 0.2-μm syringe filter. The supernatant was aliquoted into sterile 1-ml polypropylene cryopreservation vials (Fisher Scientific) and stored at −70°C until analysis.

Fresh fecal samples from emus suffering late-stage encephalitis-like illness were collected with a cotton swab. Swabs were rinsed in vials containing 0.7 ml of BFD and the vials were placed immediately on wet ice for transport to the laboratory where they were frozen at −70°C until analysis. Blood, fecal, and/or tissue samples from 33 emus were tested for the presence of arboviral agents during the 3-year study.

White laboratory mice (1–3 days old) were used for virus isolation attempts. One litter of 8 suckling mice was inoculated with each thawed, undiluted inoculum. Injections were 0.015 ml by the intracerebral and 0.03 ml by the intraperitoneal routes. Inoculated mice were observed

Table 1. Emu health and arboviral antibody isolation attempts from emu sera collected at a ranch in Volusia County, FL, 1992–94.

	Number positive/number tested[1]			
	1992	1993	1994	Totals
No. emus with encephalitis-like symptoms/approx. flock size	20/85 a	0/241 b	29/441 c	—
No. emus with a diagnosed arboviral infection/no. tested	17/42[2] a	0/36 b	22/128[3] c	39/206[4]
EEE-related mortality: no. dead/approx. flock size	12/85 a	0/241 b	5/441 b	—
HI antibody detection				
Initial serum sample[5]	11/15[2]	19/36	29/118[6]	59/169[3]
Subsequent serum sample[5]	4/4	35/76	18/73	57/153
Totals for HI antibody	15/19 a	54/112 b	47/191 c	116/322
NT antibody detection				
Initial serum sample	1/3	23/61	40/118[3]	64/182[3]
Subsequent serum sample	2/3	10/74	22/73[6]	34/150[6]
Totals for NT antibody	3/6 a	33/135 a	62/191 a	98/332

[1] Row values followed by different letters differed significantly ($P < 0.05$) in the G-test.
[2] One emu with a dual EEE/SLE, HI antibody titer.
[3] Two emus with naturally acquired antibody to SLE virus.
[4] Three emus with naturally acquired antibody to SLE virus.
[5] Initial serum sample = the first serum sample collected from an individual emu, subsequent serum sample = all additional sera samples collected from individual emus.
[6] One emu with naturally acquired antibody to SLE virus.

daily for 14 days. A 1:10 suspension of brain tissue from mice found dead during the observation period was centrifuged, filtered, and passed to an additional litter of mice by the method described above. Suspected EEE virus isolations were identified and confirmed by serum neutralization tests in Buffalo green monkey kidney cell cultures as described by Day et al. (1996).

Mosquito collections: In 1992 and 1993, mosquitoes were collected near the emu ranch in 2 dry ice-baited CDC traps (J. W. Hock Co., Gainesville, FL) set overnight at irregular time intervals. In 1994, a single mosquito collection was made by ground aspiration (Day and Carlson 1985) in vegetation surrounding pens containing emus suffering encephalitis-like illness.

Collection nets containing trapped or aspirated mosquitoes were placed on wet ice and returned to the laboratory where the mosquitoes were killed with cold and separated by species, sex, and gonotrophic condition. From 1 to 100 empty female conspecifics were pooled in a chilled Potter-Elvehjem tissue grinder containing 1.0 ml of BFD and pulverized under a laminar flow biosafety cabinet. The suspension was clarified by centrifugation at 800 × g. The supernatant was aliquoted into sterile 1.0 ml Nunc cryogenic vials (Fisher Scientific) and stored at −70°C until analysis as described in detail by Day et al. (1996).

Statistical tests: Statistical differences in EEE seroconversion rates and the health status of individual emus were tested by using unplanned tests of the homogeneity of replicates tested for goodness-of-fit (G-statistic) (Sokal and Rohlf 1981).

RESULTS

In 1992, 20 of 85 (24%) emus displayed encephalitis-like symptoms including lethargic behavior, hemorrhagic diarrhea, spastic neurological ticks including uncontrolled leg kicks and walking in circles, and death. This proportion was significantly higher ($P < 0.05$, G-statistic) than that observed in 1993 (0 of 241) and 1994 (29 of 441, 6%). Similarly, 17 of 42 (40%) emus tested in 1992 had a diagnosed EEE infection, as identified by high and persistent HI and NT titers and/or EEE virus isolation. This proportion was significantly higher ($P < 0.05$, G-statistic) than that observed in 1993 (0 of 36) and 1994 (22 of 128, 17%). Finally, EEE-related mortality in emus was significantly ($P < 0.05$, G-statistic) higher in 1992 (12 of 85, 14%) compared with that observed in 1993 (0 of 241) and 1994 (5 of 441, 1%) (Table 1). One emu bled in 1992 had

Table 2. Arboviral antibody detected in emus vaccinated against EEE/WEE virus at a ranch in Volusia County, FL, 1993 and 1994.

	1993	1994	Totals
No. of vaccinated emus tested	17	46	63
Age when first bled[1]			
H	13	10	23
J	3	36	39
A	1	0	1
No. emus vaccinated once	5	12	17
No. emus vaccinated twice	8	22	30
No. emus vaccinated three times	4	12	16
No. bled before vaccination	15	10	25
No. HI-positive emus/no. tested	5/8*[2]	2/10 ns[3]	7/18 ns
No. NT-positive emus/no. tested	5/15**[4]	6/10 ns	11/25 ns
No. emus bled after vaccination	17	46	63
No. HI-positive emus/no. tested	17/17*	6/46 ns	23/63 ns
No. NT-positive emus/no. tested	1/17**	13/46 ns	14/63 ns

[1] H = hatching (1–120 days), J = juvenile (121–365 days), A = adult (≥366 days).
[2] * = Column values for before- and after-vaccination HI antibody-positive emus differed significantly ($P < 0.05$) in the G-test.
[3] ns = No significant difference in column comparisons of similar antibody tests.
[4] ** = Column values for before- and after-vaccination NT antibody-positive emus differed significantly ($P < 0.05$) in the G-test.

a naturally acquired dual EEE/SLE infection. Two emus bled in 1994 had naturally acquired antibody to SLE virus.

In 1992, 15 of 19 (79%) emus had HI antibody to EEE virus. This was a significantly greater ($P < 0.05$, G-statistic) proportion of EEE-positive birds than was observed in 1993 (54 of 112, 48%) and 1994 (47 of 191, 25%). There was no significant difference in the proportion of NT antibody-positive emus sampled during the 3 study years (Table 1). The EEE virus was isolated from 5 of 17 (29%) emus that suffered encephalitis-like symptoms in 1992. Virus was isolated from the blood of 3 of 17 (18%) emus, 2 of which survived infection. In addition, EEE virus was isolated from the serum of one of these birds. Virus was also isolated from the brain, intestine, liver, and spleen of 2 emus that died. Fecal samples from 8 sick emus were all negative for live EEE virus. Virus isolations were not attempted in 1993 because of the apparent lack of arboviral transmission at the ranch (Table 1).

In 1994, blood or tissue from 10 of 15 (67%) emus suffering apparent EEE infection yielded EEE virus. Virus was isolated from the blood of 7 emus, 5 of which survived infection. Follow-up blood samples collected 18 days after the EEE-positive blood sample in 2 emus were negative for virus. Tissue samples were collected from 7 emus that died of encephalitis-like symptoms and EEE virus was isolated from 3; from the spleen of one, the liver of a second, and the spleen and liver of a third.

Selected emus were vaccinated against EEE virus in 1993 and 1994. In 1993, 17 (13 H, 3 J, and 1 A) vaccinated emus were monitored. Fifteen were bled at least once prior to vaccination, and all 17 were bled at least once following vaccination. In 1994, 46 (10 H and 36 J) vaccinated emus were monitored. Ten were bled before vaccination and all 46 were bled at least once following vaccination (Table 2). In 1993, 5 of 8 (62%) emus tested before vaccination had positive HI titers to EEE virus. A significantly ($P < 0.05$, G-statistic) greater proportion of the emus (17 of 17) had HI titers following vaccination. However, in 1994, there was no significant difference in the proportion of HI antibody-positive emus before and after vaccination. Likewise, there was no significant difference between pre- and postvaccination HI titers for the combined 1993–94 data (Table 2).

In 1993, a significantly ($P < 0.05$, G-statistic) greater proportion of emus tested before vaccination had positive NT antibody titers when compared with birds tested after vaccination. However, in 1994, and for the combined 1993–94 data, there was no significant difference between the proportion of emus with pre- and postvaccination NT antibody titers (Table 2).

Hatching emus from hens known to have suffered a natural EEE infection were significantly ($P < 0.05$, G-statistic) more likely to have de-

Table 3. Evidence of maternal antibody transfer to hatching-year emus at a ranch in Volusia County, FL, 1993 and 1994.

	1993	1994	Totals
No. of hatching-year emus bled before vaccination	53	72	125
No. HI-positive	22	29[1]	51[1]
No. NT-positive	24	34[2]	58[2]
HI results for chicks from hens with and without EEE antibody (No. HI-pos. chicks/no. hens)[3]			
–[3]	2/14 a[4]	8/37[1] a	10/51[1] a
+	5/13 a	10/20 ab	15/33 abc
++	10/19 a	9/12 b	19/31 b
+++	5/7 a	2/3 ab	7/10 bc
NT results for chicks from hens with and without EEE antibody: (No. NT pos. chicks/No. hens)			
–	1/14 a	13/37[2] a	14/51[2] a
+	3/13 ac	10/20 a	13/33 abc
++	14/19 b	9/12 a	23/31 b
+++	6/7 bc	2/3 a	8/10 bc

[1] One emu with naturally acquired St. Louis encephalitis (SLE) antibody.
[2] Two emus with naturally acquired St. Louis encephalitis (SLE) antibody.
[3] – = Hens without EEE antibody and with no known EEE exposure, + = EEE/WEE-vaccinated hens with no known natural EEE exposure, ++ = hens with a mild natural EEE infection, +++ = hens with a severe natural EEE infection.
[4] Column values for HI and NT antibody tests followed by different letters differed significantly ($P < 0.05$) in the G-test.

tectable HI and NT antibody titers when compared with chicks from hens with no known exposure to EEE virus (Table 3). These relationships were more evident when the 1994 hatching emus suspected of suffering a natural EEE infection (as evidenced by high, sustained HI and NT titers) were removed and the data reanalyzed. When this was done, the 1994 chicks that were progeny of hens with no known exposure to EEE virus were significantly ($P < 0.05$, G-statistic) less likely to contain HI or NT antibody to EEE virus in their first serum sample when compared with chicks from naturally infected or vaccinated hens.

Four of 140 mosquito pools tested in 1992 contained EEE virus (Table 4). Two isolates were from *Anopheles crucians* Wied.; one from a pool of 15 females collected on May 13, and one from a pool of 85 collected on May 18. The two additional EEE isolates were from pools of *Cx. erraticus* females; one from a pool of 7 collected on May 11 and one from a pool of 2 collected on May 15. No virus isolations were made from the 109 pools containing a total of 2,672 female mosquitoes representing 14 species collected in 1993. Three of 10 *Cx. nigripalpus* pools collected by ground aspiration on March 27, 1994, contained EEE virus. Two pools contained 10 females and the third contained 15 (Table 4).

DISCUSSION

Emus are susceptible to EEE infection and the virus can cause mortality. The large amounts of blood passed via oral and anal routes as infected birds approach death suggests the presence of severe internal hemorrhage with extensive liver and intestinal damage. Some infected emus succumb quickly, moving from apparent good health to death within 48 h. Some emus in late stages of an EEE infection appear to "bleed out" in a manner similar to the symptomatology reported for humans infected with Marburg virus (Preston 1994). Similar observations were reported by Brown et al. (1993) for EEE-infected emus in Georgia and by Tully et al. (1992) for emus in southeastern Louisiana. Veazey et al. (1994) reported the pathology and symptomatology of EEE infection in emus.

Our study was initiated in 1992 in response to a suspected arboviral epizootic at the Volusia County emu ranch. We isolated EEE virus from sick and dying emus on our first visit. The estimated financial loss due to EEE infection and resulting emu mortality in 1992 was $192,000.

There was no evidence (emu sickness, mortality, or viral isolation from mosquito pools) of EEE transmission at the ranch in 1993. However, emu mortality and EEE-positive mosquito pools during April of 1994 indicated that the virus was again being transmitted.

Table 4. Arboviral isolations from mosquito pools collected around an emu ranch in Volusia County, FL, 1992–94.

Species	Number EEE-positive pools/no. of pools tested (total number of mosquitoes tested)			Totals
	1992	1993	1994	
Ae. albopictus	0/3 (12)	0/1 (1)	nt[1]	0/4 (13)
Ae. atlanticus	0/10 (74)	0/1 (2)	nt	0/11 (76)
Ae. infirmatus	0/4 (31)	0/8 (118)	nt	0/12 (149)
Ae. mitchellae	0/1 (1)	nt	nt	0/1 (1)
Ae. triseriatus	0/1 (1)	nt	nt	0/1 (1)
Ae. vexans	nt	0/1 (8)	nt	0/1 (8)
An. crucians	2/15 (568)	0/17 (378)	nt	2/32 (946)
Cq. perturbans	0/8 (25)	0/8 (219)	nt	0/16 (244)
Cx. erraticus	2/17 (314)	0/18 (150)	nt	2/35 (464)
Cx. quinquefasciatus	nt	0/1 (126)	nt	0/1 (126)
Cx. salinarius	0/11 (241)	0/7 (140)	nt	0/18 (381)
Cx. nigripalpus	0/40 (2,915)	0/26 (1,370)	3/10 (105)	3/76 (4,390)
Cs. melanura	0/12 (230)	0/8 (51)	nt	0/20 (281)
Ma. dyari	0/2 (2)	0/4 (49)	nt	0/6 (51)
Ma. titillans	0/3 (3)	0/5 (24)	nt	0/8 (27)
Ps. ciliata	0/1 (1)	nt	nt	0/1 (1)
Ps. columbiae	0/7 (199)	0/4 (36)	nt	0/11 (235)
Ps. ferox	0/1 (4)	nt	nt	0/1 (4)
Uranotaenia spp.	0/4 (12)	nt	nt	0/4 (12)
Totals	4/140 (4,633)	0/109 (2,672)	3/10 (105)	7/259 (7,410)

[1] nt = No pools were tested.

Selected emus were treated with an EEE/WEE vaccine in 1993 and 1994. Some had detectable HI and NT antibody titers to EEE virus prior to vaccination (Table 2). There are at least 2 possible sources of this antibody: natural infection and maternal antibody transfer. Natural EEE infections were easy to identify because of persistent and high HI (>40) and NT (≥2.0) antibody titers. On the other hand, maternally derived antibody titers in chicks were similar to those produced by vaccination; they were low (HI = 1:10, NT < 1.6) and ephemeral. Of the 17 emus that were monitored following vaccination in 1993, 5 were suspected of having maternally derived antibody based on prevaccination serology. The remaining 12 had positive HI antibody titers resulting from vaccination. One of the 1993 emus was tested 8 days, and 4 were tested 22 days, following vaccination. All had negative HI and NT antibody titers. Four of the 1993 emus were tested 26 days after vaccination and all had positive HI (but negative NT) titers. Apparently, it takes more than 3 wk for detectable HI titers to appear in response to the vaccination protocol we used. Emus that received multiple vaccinations (up to 3 at 1-month intervals) appeared to have higher and more prolonged HI titers. Detectable antibody titers in multiply vaccinated emus persisted for at least 3 months. We did not sample vaccinated emus in a manner that allowed the calculation of antibody half-life.

In 1994, 40 of 46 vaccinated emus failed to produce detectable HI antibody titers (Table 2). This may be a reflection of the age at which emus were first vaccinated. In 1993, the mean age for first vaccination was 137 days (SD = 95.1, range = 48–323 days), whereas in 1994 the mean age for first vaccination was 91 days (SD = 21.7, range = 53–152 days). Four of the 1993 emus were older than 275 days when they were first vaccinated, and all showed positive (≥1:20) HI titers. As with horses (Gibbs et al. 1988), the age of first vaccination for emus may be important due to possible interference of maternally derived antibody with the vaccine. Emus should probably not be vaccinated earlier than 3 months of age, especially when hens have natural or vaccine-derived EEE antibodies.

In 1993 and 1994, NT antibody was more prevalent in emus prior to vaccination compared with emus following vaccination (Table 2). This is possibly due to maternal NT antibody transfer by naturally infected hens to their 1993 and 1994 offspring. It does not appear that the vaccination protocol used in our study resulted in detectable NT antibody titers in young emus.

Four hens that suffered a severe, near fatal EEE infection in 1992 survived to produce

chicks in 1993 and 1994. Maternal transfer of naturally acquired arboviral antibody from hens to their chicks is well documented (Reeves et al. 1954, Sooter et al. 1954, Bond et al. 1965). Naturally infected hens in our study were more likely than uninfected or vaccinated hens to transfer HI and NT antibody to their chicks. As in horses, the acquisition and persistence of maternally derived EEE antibody in emu chicks may influence the vaccination protocols for EEE virus. Maternally derived antibody titers (HI and NT) appeared to persist for at least 60 days and were consistently low when compared to titers in naturally infected emus.

The EEE/WEE vaccination protocol for emus needs to be thoroughly evaluated in terms of the age at which birds are vaccinated, the amount of vaccine administered, the number and frequency of booster shots required to achieve protection against arboviral infection, and the possible confounding effect of maternally derived EEE antibody titers.

The EEE virus was recovered from 3 of 19 mosquito species tested; *An. crucians, Cx. erraticus,* and *Cx. nigripalpus*. None of the 20 pools of *Culiseta melanura* (Coq.) yielded EEE virus, even though this species is presumed to be a major early-season EEE vector (Weaver et al. 1991). The EEE virus has previously been isolated from *Cx. nigripalpus* females (Wellings et al. 1972, Weaver et al. 1991). *Culex nigripalpus* is the major epidemic and enzootic vector of SLE virus in Florida (Day and Curtis 1994). This renders the isolation of EEE virus from this species in association with an exotic avian host worrisome relative to standard practices in vector control and public health, where it is assumed that *Cx. nigripalpus* is not an important link in the natural EEE cycle.

An important concern is the possibility that the introduction of ratite (emu, ostrich, and rhea) ranches into areas where arboviruses are endemic may create artificial viral foci, increasing the transmission of these pathogens to humans and domestic animals. The EEE virus is found in emu blood, but the level and persistence of viremias in infected emus remains undocumented. The extent to which emus and other ratites can act as bridge and amplification hosts in natural arboviral cycles remains unclear. With the rapid introduction of ratite ranches throughout North America, the interplay of these potential amplification hosts with native vectors and viruses is inevitable, and needs to be addressed as a possible public health issue.

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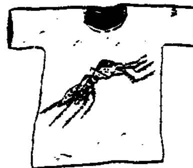

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