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SUSCEPTIBILITY OF TWO TURTLE SPECIES TO EASTERN EQUINE ENCEPHALITIS VIRUS

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Abstract: Two species of turtles collected in southern New England were inoculated subcutaneously with eastern equine encephalitis virus. The spotted turtles *(Clemmys guttata)* developed viremia and neutralizing antibody after exposure to 3 logs or more of virus. Viremia was not detected in the eastern painted turtles *(Chrysemys picta),* and neutralizing antibody was detected in only 1 of 15 inoculated *C. picta;* however, since pre-inoculation serum was not obtained from this animal, the possibility of natural infection cannot be eliminated.

INTRODUCTION

It has been proposed that eastern equine encephalitis (EEE) virus may survive the winter in the northeastern USA in a vertebrate or invertebrate host.[5] The enzootic mosquito vector overwinters in the larval stage, and those species which do overwinter as adult females generally are poor vectors of EEE virus. A naturally-infected vertebrate host has not yet been shown to carry the infection over the winter, although there is limited experimental evidence that overwintering is possible in reptiles.

Karstad[4] collected 56 reptiles (21 species) near the Okefenokee Swamp in Georgia, USA, and inoculated them subcutaneously with 1 to 3 log LD_{50} of EEE virus. Forty-four developed viremia, antibody, or both. Four to 8 log LD_{50} of viremia were commonly observed, with two animals having viremias of greater than 10 log LD_{50}. One alligator inoculated with 1 log LD_{50} circulated virus for 38 days.

Hayes *et al.*[3] experimentally infected several reptiles collected in southeastern Massachusetts with 3 log $TCID_{50}$ of EEE virus and found that 55% became viremic with amounts of virus ranging from 3.3 to 9.2 log LD_{50}, the duration of viremia being 2 to 21 days. Four animals induced to hibernate (1 garter snake, 3 spotted turtles) were viremic for 163 and 189 days.

The present study was undertaken to test the susceptibility of two species of turtles to experimental EEE virus infection.

MATERIALS AND METHODS

Seven spotted turtles *(Clemmys guttata)* and 15 eastern painted turtles *(Chrysemys picta),* collected in south-central Connecticut and southeastern Massachusetts, were held at 23 C in groups of one to six, separated by species and type of virus inoculum. Water levels in the containers were varied to allow the animals' shells to dry regularly. Once a week they were fed dried high protein dog food, small chunks of beef and, during summer months, fresh pickerel. Pre-inoculation sera were collected by cardiac puncture from most of the animals and tested in suckling mice for neutralizing antibody to EEE virus.

Three spotted turtles were inoculated subcutaneously with 1, 2, and 3 log

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suckling mouse intracerebral (SMIC) LD_{50}, respectively, of EEE strain 70357, an isolate from *Culiseta melanura* collected in Farmington, Connecticut; and four were inoculated with 4.2 log SMIC LD_{50}. The virus had been passed once in day-old chicks. The fifteen painted turtles were inoculated subcutaneously with 3 log SMIC LD_{50} of strain 70357 EEE virus.

The turtles were bled at intervals during the first 2 months post-inoculation, and the undiluted blood was tested for evidence of viremia in suckling mice by the intracerebral (i.c.) route. The level of viremia was determined by i.c. inoculation of suckling mice of serial ten-fold dilutions of virus-containing blood in phosphate-buffered saline containing 0.75% bovine albumin. Identification of virus isolations was by the complement fixation test[1] using mouse brain antigen and a reference EEE hyperimmune mouse ascitic fluid. Pre- and post-inoculation sera were tested at a 1:10 dilution (non-heat inactivated) by the intra-peritoneal route in suckling mice for evidence of neutralization of 2.2 log LD_{50} of strain 687 virus, another Connecticut isolate of eastern encephalitis virus, which was used as a first passage infant mouse brain suspension.

RESULTS

Viremia was not detected in spotted turtles receiving 1 and 2 log LD_{50}, but was found in the 5 animals inoculated with 3 or greater log LD_{50} of EEE virus (Table 1). Sera of turtles which developed viremia were positive in the neutralization test at 90 days (number 3), 122 days (numbers 4 and 7), and 178 days (number 5 and 6). Serum of spotted turtle number 2, in which viremia was not detected, was negative in the neutralization test 90 days post-inoculation.

Viremia was not detected in the painted turtles. Serum samples from one of these, taken 10 and 40 days post-inoculation, neutralized EEE virus. Pre-

TABLE 1. Detection of viremia in spotted turtles *(Clemmys guttata)* inoculated with EEE virus.

Animal number	Log LD_{50} inoculated	Pre-inoculation	Days post-inoculation											
			4	6	7	8	11	13	18	21	31	35	39	49	53
1	1.0	0*				0		0							
2	2.0			0				0		0		0			
3	3.0		0				7.2			7.5		0			
4	4.2	0	1.4								2.6	0			
5	4.2	0			2.7								0.9		
6	4.2	0					2.6							1.0	
7	4.2	0							4.5						1.1

*Log LD_{50} viremia, suckling mice, intracerebral inoculation; O = viremia not detected.

inoculation serum was not available and therefore natural infection with EEE virus cannot be excluded. Serum taken from this turtle 159 and 306 days post-inoculation did not neutralize the virus, nor did specimens from the other 14 painted turtles.

DISCUSSION

The spotted turtle is found in swampy areas such as those preferred by *Cs. melanura,* the principal vector of EEE virus in the eastern USA. Spotted turtles probably are quite common but their shy habits[2] make them very difficult to collect. The high virus titers of two animals included in this study and the lengthy duration of viremia suggest that consideration be given this species as a potential overwintering host in the natural cycle of EEE virus. Although the eastern painted turtle is the most commonly encountered turtle in southeastern New England,[2,6] the results of this small study do not implicate the painted turtle as a reservoir of EEE virus.

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