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Encephalomyocarditis (EMC) Virus Recovered From Two Cotton Rats and a Raccoon[1]

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ABSTRACT

Encephalomyocarditis in wild mammals is rarely reported. This paper documents the isolation of the encephalomyocarditis (EMC) virus in two cotton rats (*Sigmodon hispidus*) and a racoon (*Procyon lotor*) from central Florida.

INTRODUCTION

Neutralizing antibodies have been demonstrated in the sera of several rats: *Rattus rattus* and *R. alexandrinus* trapped in the United States[10], *R. rattus* trapped in Panama[11], and *R. rattus R. soridus conatus,* and *Melomys lutillus littoralis* in Australia[12]. Experimental studies have shown that certain species of rodents - the cotton rat, the hamster (*Mesocricetus auratus*)[6], the multimammate rat (*Mastomys nutalensis*)[7], the black rat[1], and the albino rat (*R. norvegicus*)[8], are susceptible to infection with EMC virus. It is the understanding of the authors that EMC virus has been isolated from only four wild animals indigenous to the United States. In the first case, the virus was recovered by the Florida State Board of Health Laboratory from the brain of a squirrel submitted for rabies examination[12]. In the second, third, and fourth cases, the virus was isolated respectively from rat feces[3], from a squirrel brain[2], and a raccoon spleen[2].

MATERIALS AND METHODS

From February 22 to 24, 1966, 14 mammals, 1 wild bird, and 1 slug were live-trapped or collected as a part of an epizootiologic investigation following an outbreak of EMC in swine near Winter Haven, Florida[4]. Sera for neutralization tests and tissues for isolation attempts were taken from 6 cotton rats, 2 rice rats (*Oryzomys palustris*), 2 raccoons, 3 opossums (*Didelphis marsupalias*), and one civet cat (*Spilogale ambervalis*).

The mammals were captured in standard box-type live traps near the hog pen. All animals were anesthetized, bled and euthanized. Serums were stored at -60C for subsequent antibody determinations. Small portions of the heart, spleen, lung, brain and intestines were removed aseptically from each animal. The tissues were placed in separate petri dishes and frozen on dry ice for 1-3 days. They were transported to the Animal Disease Diagnostic Laboratory at Kissimmee, Florida, and stored in a -60C freezer until processed. Due to shortness of time, only the intestines were tested for the presence of virus. They were thawed and ground in sterile mortars and pestles with the aid of alundum. Twenty per cent suspensions of tissue were made with brain liver heart broth* (BLHB) containing 1000 units of penicillin and 1000 mcg streptomycin per ml and 20-30% normal inactivated rabbit serum (NRS). The suspensions

* Difco Company, Detroit, Michigan.

[1] A more detailed report of these finding is to be published in the *Cornell Veterinarian*[4].

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were centrifuged at 3000 RPM for 30 minutes. The supernatant fluid was stored in 4 ml screwcap vials at -60C. Weanling mice inoculated intracranially with .03 ml suspension were observed daily for clinical signs. Deaths occurring within 24 hours after inoculation were considered non-specific. All mice dying later than 1 day after inoculation were frozen for subsequent brain harvest. Infected brain material was aseptically removed, triturated in a sterile mortar and pestle and made into 20% suspensions with BLHB and centrifuged. The EMC virus was identified by incubating decimal dilutions of this first-mouse-passage brain suspension with NRS and EMC immune serum, inoculating mice, recording deaths, and comparing LD50's or harmonic mean survival times of controls with immune serum groups.

Serums were inactivated at 56C. for 30 minutes. They were then tested in mice in a screening neutralization test against approximately 100 LD50's of virus. Mouse deaths were recorded; harmonic mean survival times (HMST's) of test serums were compared with HMST's of NRS as a control.

RESULTS

Table 1 lists the results of the findings. As indicated, no detectable antibodies were present in the serums tested. Virus was isolated from 2 of 6 cotton rats and 1 of 2 raccoons.

DISCUSSION

The isolations of EMC virus from the cotton rats is not too surprising considering the fact that other workers have found rats to: (1) possess neutralizing antibodies in nature and (2) to be susceptible to infection by EMC virus. The source of infection for the raccoon could have been from the ingestion of infected cotton rats. Kilham[9] showed that mongooses are susceptible to infection by the oral route, and speculated that they might become infected by predation upon field rats carrying EMC virus. The role, if any, raccoons may play in the continued transmission of EMC to domestic swine is unknown. But, isolation of the agent from an intestinal suspension of the raccoon indicates the raccoon could easily in the course of its daily activities, serve to maintain and disseminate EMC virus in nature.

The lack of presence of any detectable antibody in the serums of these animals yet the presence of virus in the intestine suggests: (1) that the infection was very recent, possibly from the infected water[4] on the premise, and was before antibodies had had a chance to be formed; (2) that the virus was just passing through the intestines of these wild animals without actual replication taking place. The titer of virus in the intestine of these animals was of a low level, near the fifty per cent end-point for mice[4].

Table 1. *Serum Neutralization Tests and EMC Virus Isolations on Samples Collected February 21-24, 1966*, Winter Haven, Florida*

Host	Serum Neutralizations			Virus Isolation Attempts	
	No. Taken	No. Tested	No. Positive	No. Tested	No. Positive
Cotton Rat (*Sigmodon hispidus*)	6	4**	0	6	2
Rice Rat (*Oryzomys palutris*)	2	2	0	2	0
Opossum (*Didelphis marsupialis*)	3	3	0	3	0
Raccoon (*Procyon lotor*)	2	2	0	2	1
Civet Cat (*Spilogale ambervalis*)	1	1	0	1	0
Slugs (*Deroceros agriolimoz*)	1	NA	--	1	0
Boattailed Grackle (*Cassidix mexicanus*)	1	1	0	NT	--
Totals	16	13	0	15	3

NA - not applicable; NT - not tested
* Portion of Table 3[4].
** Two were not tested due to insufficent serum.

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