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SEROLOGIC EVIDENCE OF VENEZUELAN EQUINE ENCEPHALITIS IN SOME WILD AND DOMESTIC POPULATIONS OF SOUTHERN TEXAS

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Abstract: More than 2,500 sera from approximately 30 wild and domestic species in southern Texas were tested for neutralizing antibodies to Venezuelan equine encephalitis (VEE). Virus isolations were also attempted from blood and tissue samples of many of the wild specimens. VEE neutralizing substances were present in a variety of species collected prior to the 1971 epizootic, suggesting that VEE was present and perhaps enzootic in this area before the recent epizootic. Serologic results of this study suggest that deer (*Odocoileus virginianus*) and feral swine (*Sus scrofa*) may serve as good indicators or sentinels of VEE transmission. The reservoir of VEE was not established, but results of the study suggest that a number of species or a combination of animal host populations including deer, feral swine, and peccaries (*Pecari angulatus*) may be involved in the epizootiology of VEE in southern Texas.

INTRODUCTION

Venezuelan equine encephalitis is caused by a zoonotic alpha virus (group A arbovirus) which can infect man as well as wild and domestic animals.[1] Results of studies in Central and South America as well as Florida suggest that wildlife species, especially rodents, might be involved in the epizootiology of VEE.[7,13,17]

The 1971 epizootic of VEE which extended from Central America into Texas demonstrated the potential importance of this disease to equine and human populaitons as well as illustrated our lack of knowledge about the ecology of VEE in the United States. Results of serologic studies in Texas prior to and during the 1971 epizootic suggested wildlife involvement;[5,20,21] therefore, in 1972 research was initiated at the Rob and Bessie Welder Wildlife Refuge to increase our knowledge of the ecology of VEE in Texas. This study site, 18 km northwest of Corpus Christi, was selected because of its history of wildlife disease research and location in the 1971 epizootic area.

Specific objectives of the study were to: 1) determine the presence and prevalence of VEE in wild, domestic, and sentinel animal populations on the Refuge, 2) evaluate the role of wildlife in the epizootiology of VEE and 3) study potential vector populations on the Refuge.

MATERIALS AND METHODS

Vertebrate sera used in the serologic study were either a) collected prior to 1971 as part of other studies[5,11] or b) collected specifically for this study during 1972 and 1973. Virus isolation attempts were made from blood and tissue specimens from animals collected in 1972 and 1973. Wild animals were captured using a variety of methods, i.e., Sherman and Hav-a-hart live traps, drop nets, box traps, shooting. Blood was obtained from live animals by cardiac

puncture or venipuncture. Blood, serum, and tissue specimens from dead animals were frozen at —65C and shipped on dry ice to the Veterinary Science Laboratory at the University of Wisconsin—Madison to test for virus content.

An estimate of mosquito populations at various locations on the Refuge was based on the number of mosquitoes aspirated from human bait. Collections were made at each sentinel hamster site for 20 min between 2000 and 2300 hours, once a week during June, July, and August. Mosquitoes were identified to genus, placed in sterile vials, frozen and shipped to the laboratory for virus analysis. Rainfall data on the Refuge were compiled from records maintained by W. C. Glazener, Director at the Welder Refuge.

A hamster (*Cricetus cricetus*) sentinel system was established to monitor VEE virus transmission on the refuge during June, July and August of 1972 and 1973. Three sentinel sites, which transected the Refuge and represented possible "VEE mosquito" areas, were selected. Three hamsters of different color were placed at each of the sites in a 1.25 cm wire mesh cage which was situated approximately 15 cm off the ground. Hamsters were examined daily for signs of disease.

A domestic hog sentinel system was established in the spring of 1973 to complement the hamster sentinel system. Two hogs were confined to large (approximately 3 x 9 m) wire pens in close proximity to each of the three hamster sites. The hogs were examined daily for signs of disease. Blood samples for virus and antibody testing were collected weekly from at least one hog and one hamster at each sentinel site.

All serum samples were heat-inactivated at 56C for 30 min and tested for the presence of neutralizing antibody to VEE virus in the HeLa cell metabolic inhibition test (MIT).[11] To test for intragroup cross neutralization, Western equine encephalitis (WEE) virus was also included in the test.

Blood and tissue specimens from sentinels, captured animals, and mosquitoes were emulsified in 10% suspension and assayed for virus. All viral assays were done in either a standard plaque test with a HeLa cell line, or in 2-4-day-old suckling mice inoculated intracerebrally.[10]

RESULTS

More than 2,600 sera from reptiles, birds, and mammals were tested for VEE and WEE virus neutralizing antibody in the MIT. Sera from a variety of wild and domestic mammal species on the Welder Refuge neutralized VEE virus (Table 1). Numerous sera collected prior to 1971 had antibody, including deer, cattle (*Bos taurus*), rabbits (*Sylvilagus floridanus*), feral hogs, pecaries, and pocket gophers (*Geomys bursarius*).

Of particular interest were the results in feral hog and white-tailed deer populations. Feral hog populations had high antibody prevalence rates during the study period. Deer sampled during each year of the study had VEE antibody with the highest prevalence rates occurring in 1967, 1969, 1971, and 1973. The age- and sex-specific VEE antibody prevalence rates were compared using the chi-square test. These rates increased with age from 12% (9 of 78) of fawns tested to 34% (51 of 151) adult deer 2 years and older. This difference in age-specific antibody prevalence is significant (PS $\leq$.01). Antibody prevalence in female deer was 31% (49 of 160) and the prevalence in males was 19% (22 or 117).

There was little or no antibody detected in sera from more than 250 rodents, 75 raccoons (*Procyon lotor*), 30 opossum (*Didelphis marsupialis*), and 126 coyotes (*Canis latrans*), although these species were reported to be involved in VEE epizootiology in other studies.[3,7,13,17] VEE antibody was detected in less than 2% of more than 1,000 turkey (*Meleagris gallopavo*) sera examined and in none of the sera from 87 black (*Coragyps atratus*) and turkey vultures (*Cathartes aura*) tested. Antibody was detected in one of the seven rattlesnake (*Crotalus atrox*) sera tested.

TABLE 1. Summary of antibody prevalence to Venezuelan equine encephalitis virus for selected mammal species at the Welder Wildlife Refuge (1963 - 1973).

Host	Prior to 1971	1971	1972	1973
Cattle *Bos taurus*	50/116 (43)*	15/41 (37)	7/10 (70)	....
Goat *Capra hircus*	4/6 (67)	....	....	....
Horse *Equus caballus*	0/54 (0)	....	....	....
Sheep *Ovis aries*	....	....	0/2 (0)	....
Feral Hog *Sus scrofa*	3/7 (43)	....	19/32 (59)	9/12 (75)
White-tailed Deer *Odocoileus virginianus*	91/331 (28)	29/70 (41)	12/48 (25)	16/45 (36)
Peccary *Pecari angulatus*	12/30 (40)	....	....	....
Raccoon *Procyon lotor*	3/21 (14)	0/1 (0)	0/1 (0)	0/52 (0)
Opossum *Didelphis marsupialis*	0/20 (0)	....	0/3 (0)	0/7 (0)
Striped Skunk *Mephitis mephitis*	....	....	0/3 (0)	....
Armadillo *Dasypus novemcinctus*	....	0/1 (0)	1/3 (33)	0/11 (0)
Coyote *Canis latrans*	2/124 (2)	....	....	0/2 (0)
Bobcat *Lynx rufus*	0/9 (0)	....	....	....
Cotton-tailed rabbit *Sylvilagus floridanus*	3/8 (38)	....	0/1 (0)	....
Pocket Gopher *Geomys bursarius*	6/31 (19)	....	....	0/7 (0)
Pack Rat *Neotoma micropus*	0/70 (0)	....	....	0/12 (0)
Black Rat *Rattus rattus*	....	....	....	0/2 (0)
Pygmy Mouse *Baiomys taylori*	....	....	0/142 (0)	0/75 (0)
Harvest Mouse *Reithrodontomys megalotia*	....	....	0/1 (0)	0/5 (0)
White-footed Mouse *Peromyscus leucopus*	....	....	0/3 (0)	0/7 (0)
House Mouse *Mus musculus*	....	....	....	0/1 (0)

* No. sera neutralizing virus / total area tested: ( ) = percent antibody prevalence.

The possibility of cross neutralization between two group A arboviruses or dual infections in the specimens tested was considered and all sera were tested for WEE and VEE. Only cattle sera (63 of 88; 75%) and peccary sera (8 of 12; 66%) neutralized both VEE and WEE in substantial numbers.

There was no serologic or virological evidence of VEE virus transmission to the sentinel hamsters or to the sentinel hogs.

No virus isolations were made from tissue, blood or mosquito specimens summitted for laboratory examination during 1972 and 1973.

Six genera of mosquitoes including *Psorophora, Culex, Aedes, Culiseta, Mansonia,* and *Anopheles* were collected on the Welder Refuge at various times during 1972 and 1973. The most prevalent genera captured were *Psorophora* (65%) and *Culex* (29%). Results of weekly mosquito surveys indicated that vector abundance was related to rainfall.

DISCUSSION

It is appreciated that serologic test results must be interpreted cautiously in the absence of virus isolations, or the identification of neutralizing substances in immunoglobulins. The specificity and sensitivity of the MIT for all species tested have not been completely evaluated; however, where comparative quantitative and qualitative results are available, the reliability of the MIT has been good.[5,6] Experimental studies on selected animal species such as deer have also been done and expected antibody responses were verified.[9] To examine the specificity of VEE virus neutralization, WEE virus antibodies were also tested for, and in most species neutralization was apparently specific for WEE or VEE.

Based on MIT results, a number of wild and domestic species on the Welder Wildlife Refuge including cattle, feral hogs, peccaries, deer, raccoon, pocket gophers, rabbits, and coyotes had been infected with VEE prior to the 1971 epizootic. These findings and the results of previous studies[5,11] suggest that VEE was present and possibly enzootic in Texas for many years prior to the 1971 epizootic; a situation which may be similar to that in Florida.[2,3,4]

Scherer *et al.*[13] stated that in Mexico, sera from cattle and pigs were frequently positive for VEE prior to the 1970 epizootic of the IB subtype. Cattle and hog serologic results from the Welder Refuge indicate a similar situation, although cross reactions may have occurred in cattle sera, making the VEE results questionable.

In the deer population, the VEE virus antibody prevalence rate fluctuated annually. The higher VEE antibody prevalence among adult than fawn deer may simply be the result of older deer having had greater opportunity for VEE exposure. Also, maternal antibodies in young fawns may limit their susceptibility to VEE infection.[12] Since deer collections occurred primarily during the winter and spring months, very few sera from young fawns (1-3 months) were tested. Most fawn sera were from deer 6-12 months of age and maternal antibody would not be detectable at this time.

Because of their susceptibility to infection and relative east of sampling, deer appear to be a good species to monitor VEE virus transmission—a wildlife sentinel.[8]

VEE prevalence data of deer in this study differed slightly from prevalence data of earlier reports.[5,11] This was due to the fact that the year recorded as the date of serum collection was adjusted to the potential transmission season. For example, deer sera collected in January of 1972 would have been recorded as 1971, since any transmission would have occurred during the summer of 1971.

A high percentage (60%) of the feral hog sera tested during this study had VEE antibody. These findings, along with results of experimental studies in hogs[9] suggest that feral hogs may serve as indicator hosts and a possible reservoir of VEE. The lowland habitat of the feral hog would be conducive to a hog-mosquito-hog VEE cycle.

It has been reported that rodents act as silent hosts of enzootic strains of VEE

in Central and South America.[7,13,19] Results of this study do not incriminate rodents as significant reservoir species in the long-term maintenance of VEE in southern Texas.

Other studies have suggested that the opossum,[7] rabbit,[8] coyote,[16] and vulture,[7] may have been involved in a recent epizootic of the IB subtype of VEE virus. The opossum[14] and raccoon[2] were also incriminated as possible disseminating hosts of VEE virus in Florida. Results of this serologic study would not incriminate these species in the epizootiology of VEE in southern Texas.

Hess[8] reported a high VEE virus antibody prevalence rate in rabbits from Texas, and Mackenzie[16] suggested that VEE epizootics may cause high mortality in rabbits. Serologic results of this study suggest that the rabbit may be important in the epizootiology of VEE but the number sampled was small.

The need for additional research to clarify the status of VEE in wildlife is obvious. Appropriate laboratory testing is needed to confirm the specificity of VEE reactors in the MIT and to identify the strain of VEE in these involved animal populations. Isolation of VEE virus from wild populations is essential to confirm VEE activity. The yearly collection of deer on the Welder Refuge may serve as a good opportunity to monitor VEE in this region of Texas. Several other animal populations such as the rabbit and peccary populations are suspect and should be studied in greater detail.

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