A SEROLOGICAL AND VIROLOGICAL STUDY OF VENEZUELAN EQUINE ENCEPHALITIS IN ANIMAL POPULATIONS OF SOUTH TEXAS

BY

DAVID L. SMART

A thesis submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

at the

UNIVERSITY OF WISCONSIN-STEVENS POINT

COLLEGE OF NATURAL RESOURCES

1974

APPROVED BY THE GRADUATE COMMITTEE OF:

____

Dean, College of Natural Resources and Advisor

____

Professor of Forestry and Natural Resources

____

Professor of Wildlife

____

Associate Professor of Biology

Date thesis is presented________________________

ACKNOWLEDGMENTS

I wish to give sincere thanks to my major advisor, Dr. Daniel O. Trainer, for his assistance in all phases of my graduate program. His guidance, understanding, participation, and moral support in this research project is gratefully appreciated.

I am indebted to fellow researcher Craig A. Wickman, for his field assistance in the capture and bleeding of animals. His companionship during this study is also appreciated.

I also wish to give special thanks to Mr. W. C. Glazener, Assistant Director of the Welder Wildlife Foundation, for his assistance in establishing the animal sentinels and general supervision of field work on the Refuge.

I also wish to acknowledge the following group who either collected sera prior to my research or who aided in the collection of animals for serum samples during my research: Drs. R. Cook, F. Knowlton, M. White, W. Samuel, J. Hoff, D. Delnicki, W. Faatz, and D. Trainer and Messrs. W. Glazener, C. Wickman, W. Foreyt, J. Kimbrough, N. Smith, G. Merrill, J. Kie, K. Cardwell, D. Mock, J. Folse, W. Reagan, D. Bliss and R. Shirer.

Dr. T. M. Yuill and Mr. B. D. Nassif, Department of Veterinary Science, University of Wisconsin-Madison, were responsible for the serologic and virologic laboratory testing phase of this research.

I am indebted to Dr. Clarence Cottam, Director of the Welder Wildlife Foundation, for his guidance and to the Welder Wildlife Foundation for their financial assistance. I would also like to thank the Welder staff for their cooperation and assistance in the field aspects of my research.

Dr. C. Long, Dr. R. Anderson, Dr. J. Newman and Mr. E. Gluesing deserve recognition for their editorial assistance in writing this thesis.

I would also like to express my deepest gratitude to my parents for their encouragement and support throughout my years in school.

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. Virus isolations were also attempted from blood and tissue samples of many of the wild specimens. VEE reactors were present in a variety of species collected prior to the 1971 Texas 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 hogs (*Sus scrofa*) may serve as good indicators or sentinels of VEE activity. The reservoir of VEE was not established, but results of this study suggest that a number of species or a combination of animal host populations including deer, feral hogs, and peccaries (*Pecari angulatus*) may be involved in the epizootiology of VEE in South Texas. Vector abundance and *Psorophora*-*Culex* prevalence patterns were shown to be related to rainfall.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Venezuelan equine encephalitis (VEE), an often fatal disease of horses, has been studied in great detail in many countries south of the United States. The purpose of this study was to learn more about the ecology of VEE in the United States, particularly Texas.

Venezuelan equine encephalitis (VEE) is a zoonotic group A arbovirus which can infect man as well as wild and domestic animals (Andrews and Pereira, 1967). The first reports of VEE were from the Columbian-Venezuelan border in 1930 (Young, 1972). VEE virus was first isolated and described by Kubes and Rios (1938) in 1938, although epizootics probably caused by the same agent had occurred in Columbia before this time. Since the original detection of this disease, epizootics have occurred in Columbia (Groot et al. 1959), Peru (Madalengiutia et al. 1972), Ecuador (Gutierrez et al. 1972), Trinidad (Gilyard, 1945), Panama (Grayson and Galindo, 1968), Guatemala (Scherer et al. 1972), Honduras and British Honduras (Scherer et al. 1970), Nicaragua (Walton et al. 1972), Mexico (Gonzalez, 1969) and the United States (Bigler, 1971; Sudia and Newhouse, 1971).

There are currently 4 major types of VEE virus with Type I divided into 5 major subtypes; all types have been detected in various locations in South, Central and North America (Young and Johnson, 1969). The epizootic subtypes, IA, IB, and IC, are highly virulent for equines, which serve as an amplifying host for the virus. The enzootic types, II, III, and IV, and the subtypes, ID and IE, produce disease in humans and in some rodents but no clinical disease in equines. The mosquito serves as a vector for all types of VEE.

Human infections of VEE may result in clinical illness with symptoms such as headache, fever, mylagia, vomiting, chills, sore throat, diarrhea, dizziness, and eye pain; mortality occurs infrequently (Barker et al. 1973). Infected horses may have signs similar to those described for humans. In addition, there can be involvement of the central nervous system resulting in the formation of lesions, particularily in the cerebral cortex (Monlux and Luedke, 1973). Mortality varies with the virus subtype, but generally occurs in approximately 30 percent of the horses infected; morbidity may be as high as 80 percent.

Other domestic and wild vertebrates seem to be relatively unaffected by VEE infections. No epizootic has been reported where significant mortality occurred in wild or domestic vertebrates, excluding equines. There has been evidence that vertebrates other than equines may serve as reservoirs or silent hosts for enzootic VEE. A rodent-mosquito-rodent cycle has been implicated in the maintenance of VEE virus in Trinidad (Jonkers et al. 1968), Panama (Grayson et al. 1968), and several other South and Central American countries and in Florida as well. The enzootic form of this disease is typically found in moist, tropical areas, although reactors to VEE have been detected in drier regions such as West Central Utah (Thorpe et al. 1965).

Investigations since 1943 in South, Central and North America have provided evidence of VEE virus and/or antibodies in approximately 72 species of mammals (USDA, 1973). Many of these mammals such as the coyote (Canis latrans), hog (Sus scrofa), and deer (Odocoileus virginianus), when experimentally infected with VEE virus, develop sufficient levels of viremia to infect vector mosquitoes and thus may function as silent hosts.

Results of experimental studies with cardinals (Richmondena cardinalis), sparrows (Zonotrichia albicollis), and domestic pigeons (Columbia livia), suggested that although birds have lower viremias than mammals, they may be involved both in the maintenance of enzootic infection and as foci for epizootic spread (Chamberlain et al. 1956). Approximately 25 bird species have been incriminated with VEE since investigations began in 1955 (USDA, 1973). Most studies indicate however, that birds are not important in the epizootiology of VEE.

Until 1971, VEE was reported only from Central and South America and in South Florida (Young and Johnson, 1969). In the spring of 1971, however, a highly virulent subtype of VEE (IB) entered South Texas and spread up the coast causing widespread morbidity and mortality among equines. Approximately 1500 equine deaths were reported by the USDA (USDA, 1972) (Figure 1). There were 88 confirmed cases of human illness contributed to VEE (USDA, 1972). This epizootic apparently originated in Ecuador in 1969, spread to Guatemala, and then to southern Mexico in 1970 causing widespread mortality among horses as well as morbidity and limited mortality in humans (Spertzel and McKinney, 1970).

VEE was detected in the United States on June 23, 1971 (Spertzel, 1971). An attenuated vaccine of tissue origin, TC-83, had previously been developed by the U. S. Army Medical Research Institute of Infectious Diseases at Fort Detrick, Maryland. On June 25, 1971, two days after the first diagnosed equine case of VEE in Texas, the vaccine was made available to horse owners in the United States on a voluntary basis (Omohundro, 1972). Additional control measures were initiated. On July 13, a quarantine was imposed on equines. Between July 11 and August 15, the USDA conducted aerial spray operations. Approximately

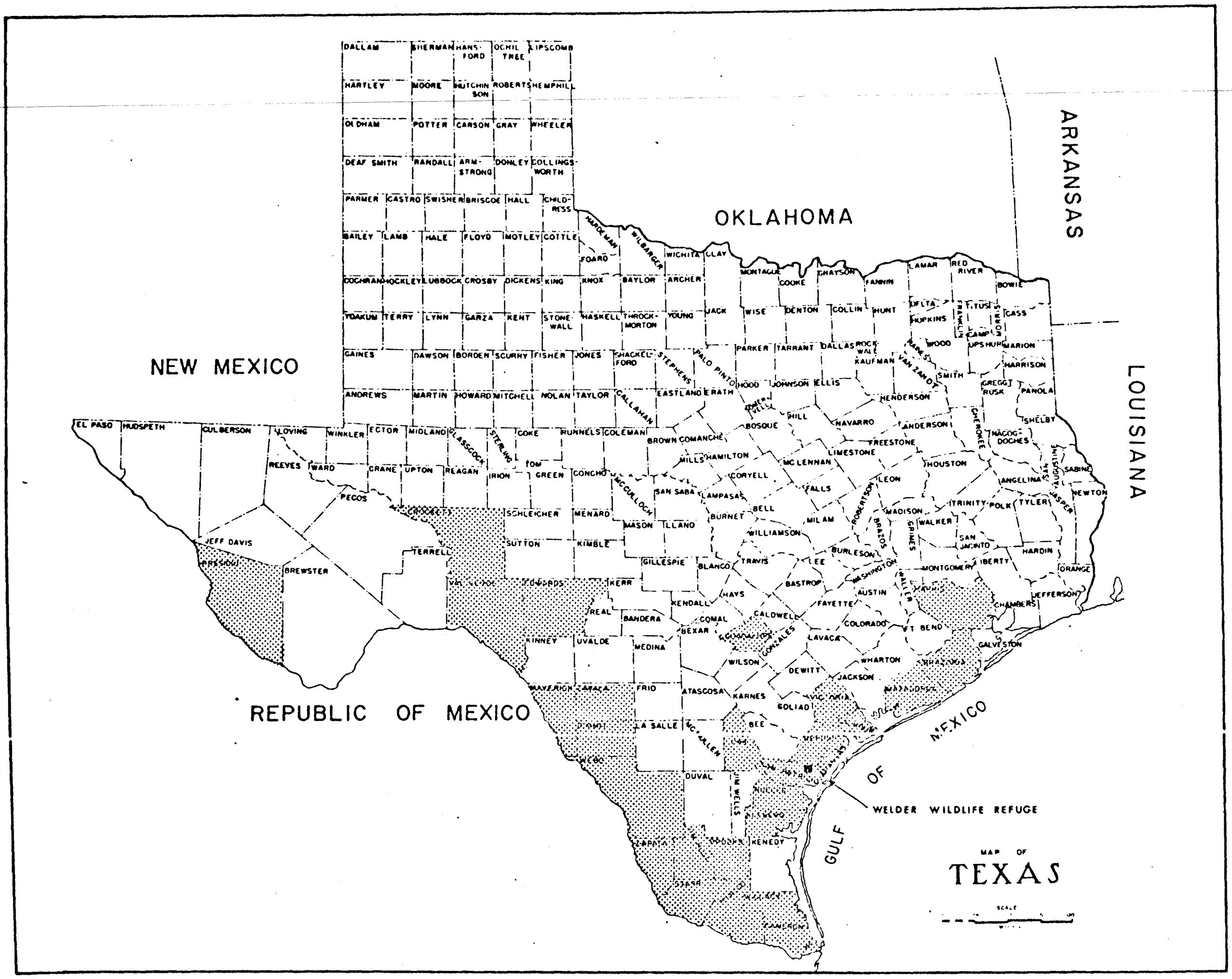

Figure 1. Location of the Welder Wildlife Refuge and counties with confirmed equine and human VEE virus in Texas, 1971 (Parker et al. 1973).

10 million acres of the Gulf Coast of Texas and adjacent portions of Louisiana were sprayed with ultra low-volume malathion. The presence, prevalence, and movement of the disease was kept under constant surveillance.

The remarkable geographic advancement of VEE despite intensive control measures, led to numerous studies in Central and South America to determine possible reservoirs or silent hosts. Results of these studies suggested that wildlife species, especially rodents, may be involved in the epizootiology of VEE (Grayson and Galindo, 1968; Jonkers et al. 1968; Grayson and Galindo, 1969; Sherer et al. 1971; Scherer et al. 1971).

At present, only Psorophora confinnis and Aedes taeniorhynchus are proven vectors of the IB strain of VEE (Sudia, 1971). Vector studies done by the Arbovirus Ecology Laboratory, Arbovirology Unit, Center for Disease Control, Atlanta, Georgia, suggested that at least 11 mosquito species may have been involved in the 1971 outbreak in Texas (Sudia and Newhouse, 1971). Mosquitoes of the genus Culex are the only mosquitoes proved to be an efficient natural vector of enzootic VEE (Galindo, 1971). An ever increasing number of mosquito species have been suggested as potential VEE vectors as entomological investigations into the ecology of VEE continue.

The 1971 epizootic of VEE in Texas documented the importance of this disease to equine and human populations and demonstrated our lack of knowledge about the ecology of VEE in the United States. Results of limited serologic studies in Texas prior to and during the 1971 VEE epizootic suggested possible wildlife involvement (Cook et al. 1965; Trainer and Hanson, 1969; USDA, 1973). As a result, research was initiated to investigate the ecology of VEE at the Rob and Bessie Welder Wildlife Foundation (Refuge). This study site was selected because of its location in the 1971 epizootic area and its history of wildlife disease research. Specific objectives of this investigation were to:

1) study wild, domestic and sentinel animal populations at the Foundation to determine the presence and prevalence of VEE in the respective populations.
2) evaluate the possible role which wildlife may play in the epizootiology of VEE.
3) study potential vector populations on the Foundation.

The Study Area

The Rob & Bessie Welder Wildlife Foundation is located approximately 6 miles inland from the southern Gulf Coast of Texas in San Patricio County (Figure 2). A substantial number of VEE cases were reported in San Patricio County in both humans and horses during the 1971 epizootic. The VEE study area included the entire 7,800 acre refuge (Figure 3).

The climate of the Welder Refuge is characterized by hot humid summers with minimum daily temperatures frequently above 80°F and relative humidity above 80 percent; winters are mild. Rainfall averages 30-40 inches annually and is sporadic. Standing water, which fluctuates extremely, occurs in the form of lakes, ponds, and temporary pools (Figure 4). Tropical storms are not uncommon to the area. The Refuge is bordered on the north by the Aransas River which occasionally overflows its banks.

The Welder Refuge is at the junction of the northern prairies, the live oak regions along the Coast, the South Texas plains, and the Gulf prairies and marshes (White, 1967). More than 1300 species of plant life, including at least 15 plant communities, are supported on the Refuge's various soil types (Box and Chamrad, 1966) (Figure 4).

The 12.2 square mile refuge supports some 55 species of mammals, 35 species of reptile, 15 species of amphibians

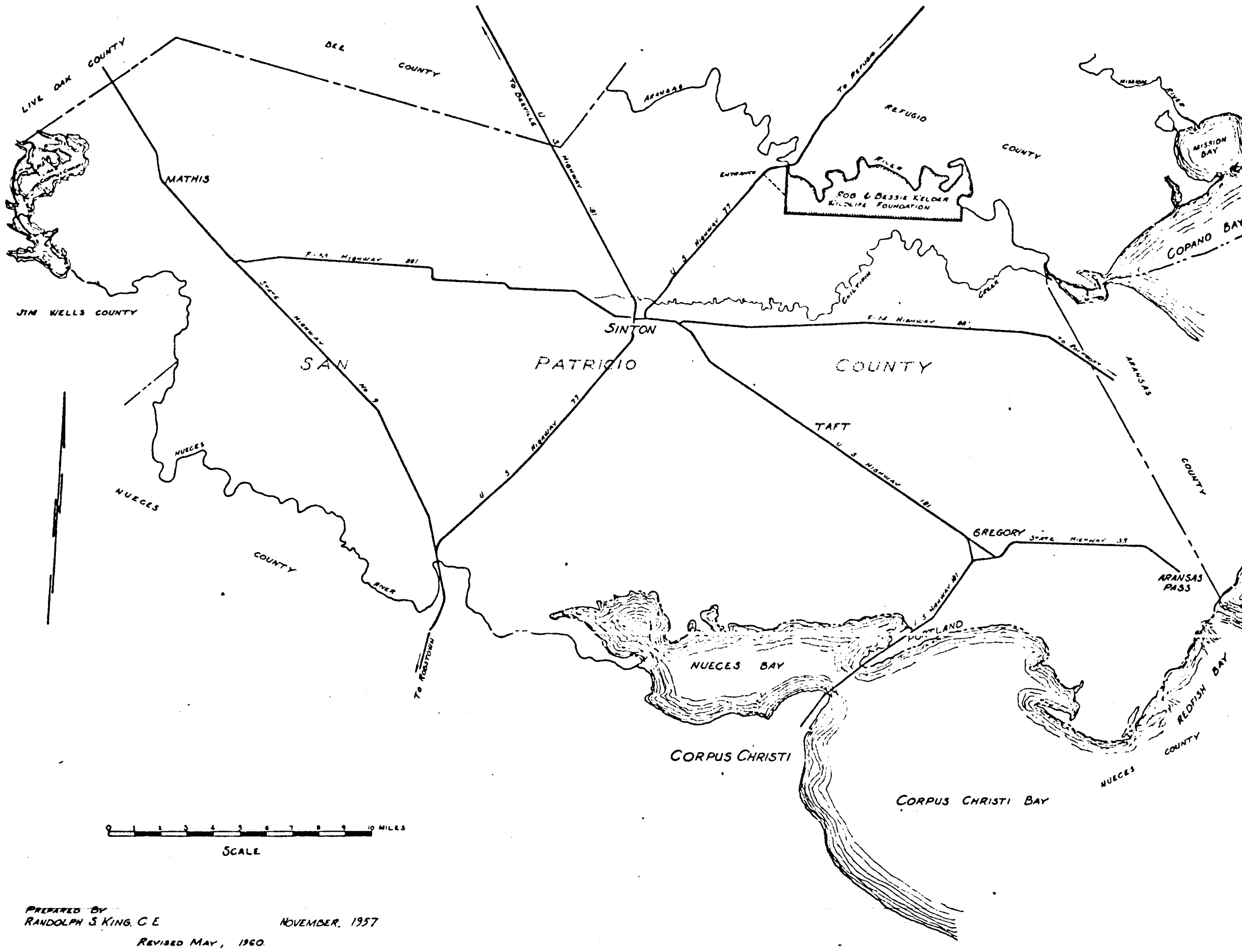

Figure 2. Location of the Welder Wildlife Foundation in San Patricio County.

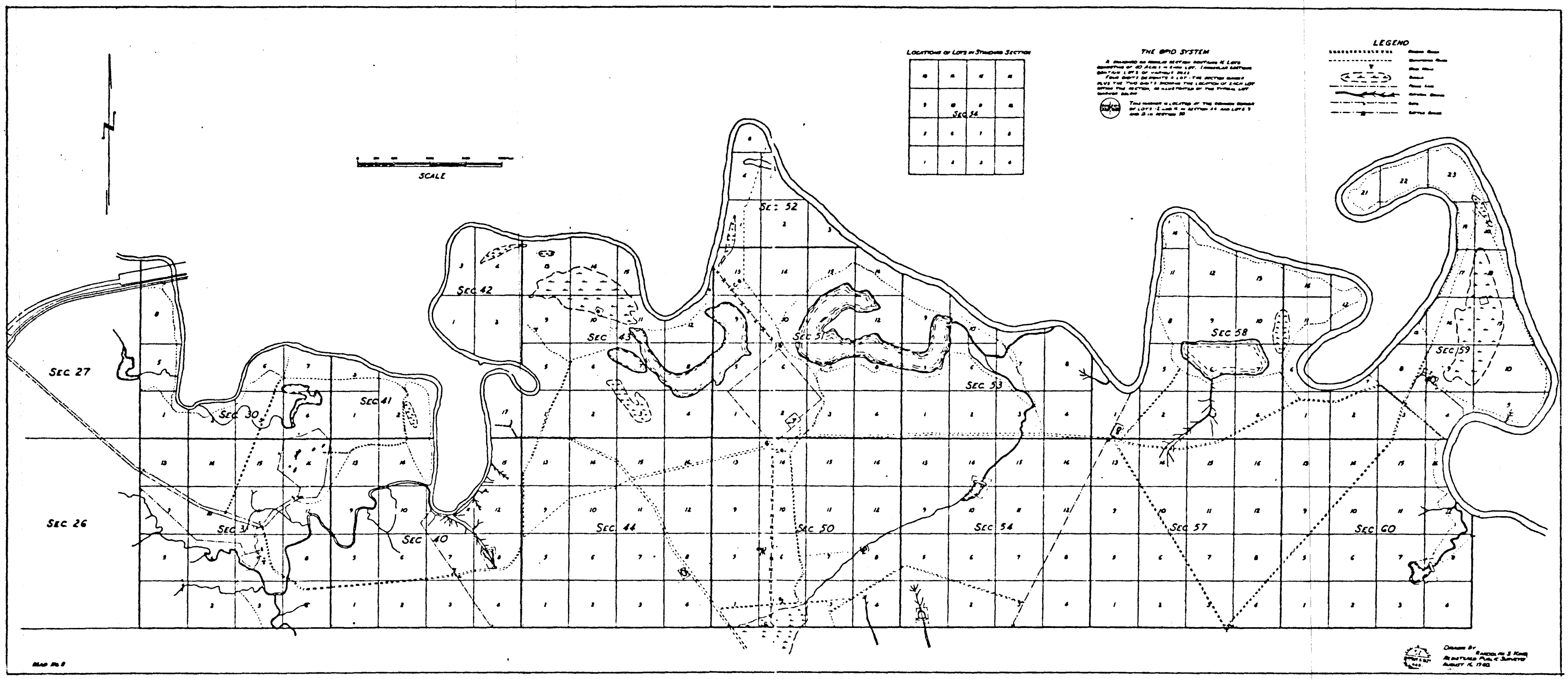

Figure 3. The Rob & Bessie Welder Wildlife Foundation.

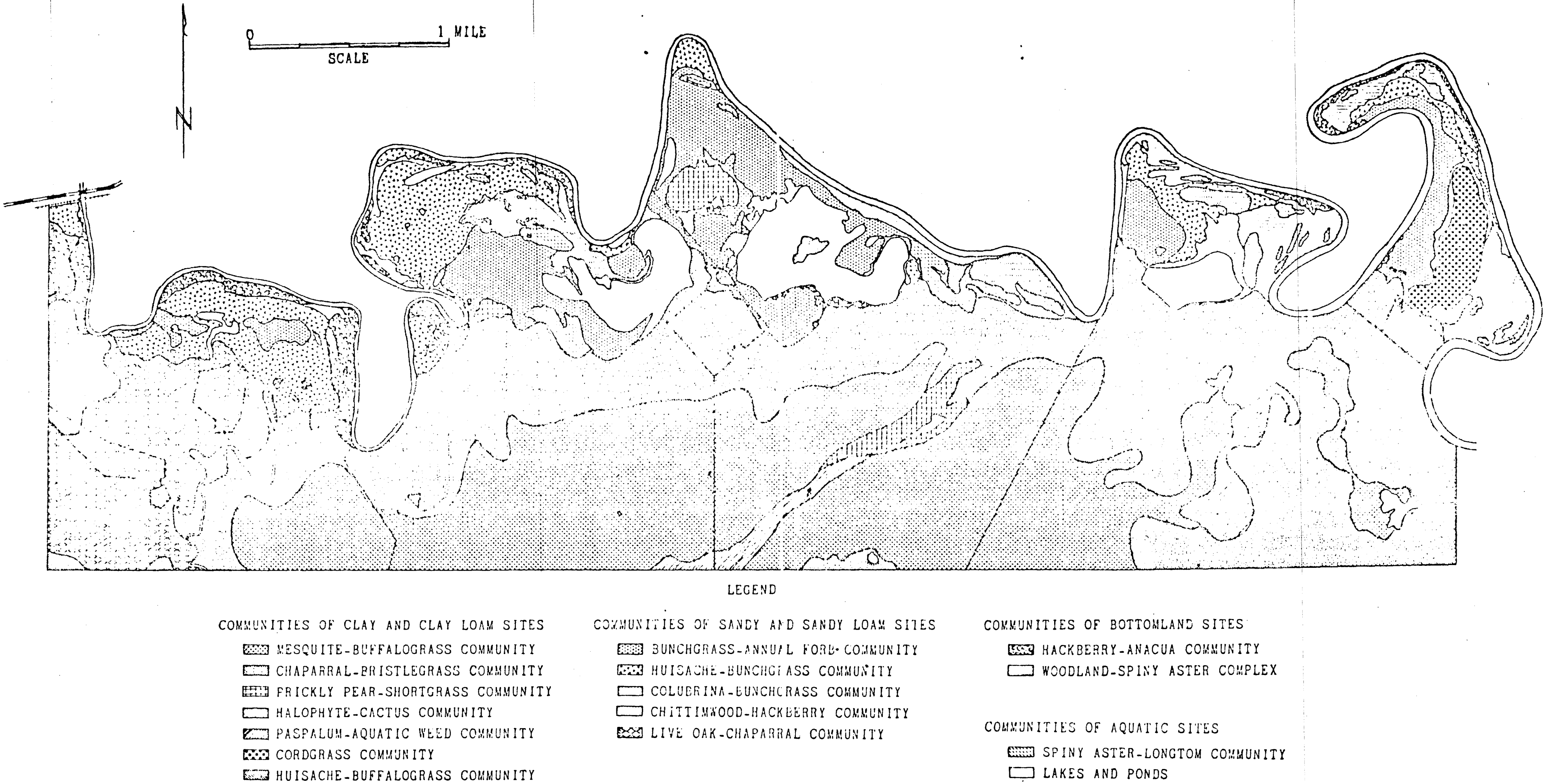

Figure 4. Plant and aquatic communities of the Welder Wildlife Refuge (Box and Chamrad, 1965).

and more than 400 species of resident and non-resident birds (Glazener, 1973). Approximately 35 species of mosquitoes, some of which are known VEE vectors, have been identified on the Refuge (Hubert, 1973).

In the summer of 1973, the deer population on the Refuge was estimated to be approximately 100-125 deer per square mile (Kie, 1973). The deer population is relatively stable and sedentary; their range seldom exceeds one square mile (Michael, 1965). Approximately 50-100 deer are collected annually for scientific purposes.

Individual small mammal populations on the Refuge are known to fluctuate annually but not necessarily in synchrony (Otteni, 1971).

The Welder Refuge has had a long history of livestock use with approximately 18 horses and 500 cattle present on the area at all times.

METHODS

Capture Methods

Vertebrate sera collected as part of other studies prior to 1972 and specifically for this study in 1972 and 1973 were examined serologically to study animal exposure to VEE on the Welder Refuge. In addition, virologic tests were conducted on blood and tissue specimens from animals collected during this study.

Wild animals were captured using a variety of methods. Small mammals were sampled utilizing Sherman live traps; larger mammals were sampled using various sized Hav-a-hart live traps (Figure 5). Various plant communities were sampled to determine the kinds and numbers of mammals present. Live traps were placed in those communities which supported the largest number of individual animals. Live traps were also located in close proximity to sentinel hamster sites in an attempt to sample specific wildlife populations. Different baits were tested to determine which were the most effective in capturing a given species of animal. Cracked corn (*Zea mays*), whole kernel corn, and apple (*Malus* sp.) proved to be most attractive to mammals while offering the least potential for insect and meteorological destruction.

Other animals were captured by using established capture methods such as utilizing drop nets and cannon nets for

Figure 5. Live traps used to capture small mammals at the Welder Wildlife Refuge. Large, medium, small- from left to right.

turkeys (Meleagris gallopavo), walk-in funnel traps for vultures (fam. Cathartidae), shooting for deer, and modified deer traps for feral hogs and peccaries (Pecari angulatus) (Figure 6). More than 7,600 trap nights were expended in trapping wild animals by a combination of these methods in 1972 and 1973. Captured mammals were identified utilizing descriptions by W. B. Davis (1960).

The presence and prevalence of mosquito populations at various locations on the Refuge were based on the number of mosquitoes aspirated from human bait. Collections were made at each hamster site for 20 minutes between the hours of eight and 11 pm, once a week, unless weather conditions altered the schedule. Mosquitoes were identified to genus, placed in sterile vials, and kept frozen until being shipped to the laboratory for virologic testing. Appropriate biological data were recorded for each pool of mosquitoes collected.

In addition to collecting adult mosquitoes, permanent and temporary bodies of water were examined for the presence of mosquito larvae. Species of larvae, date, and location were recorded. Mosquitoes were identified using descriptions by Carpenter and LaCasse (1955), the U. S. Army Medical Entomology Handbook (1972) and Pennak (1953).

Rainfall data on the Welder Refuge was compiled by W. C. Glazener, Assistant Director of the Welder Refuge.

Figure 6. A modified deer trap, used to capture feral hogs.

Collection of Serologic and Virologic Specimens

Blood was obtained from live animals via either the heart, jugular vein, brachial vein, or anterior vena cava using a needle and syringe (Figures 7 & 8). Locality, sex, age, date, species of animal, and any unusual condition was recorded for each blood and tissue sample. Blood was placed in sterile vials, allowed to clot, centrifuged, the serum decanted and frozen until tested. In 1972, all animals that were bled were also swabbed orally. Oral swabs were placed in sterile vials containing a liquid diluting nutrient mixture and frozen until tested. This procedure was deleted in 1973. Tissue samples (spleen) from sick or dead animals were removed aseptically, placed in sterile vials, and frozen until tested. Blood, selected tissue specimens, oral swabs, dead hamsters and sera were shipped on dry ice to the Veterinary Science Laboratory at the University of Wisconsin-Madison where serologic and virologic tests were conducted.

Monitoring of VEE

Penned animals have been utilized successfully to monitor zoonoses (Srihongse et al. 1967; Trainer, 1973) and hamsters (*Cricetus cricetus*) have been successfully used as sentinels to detect VEE (Scherer et al. 1964). A hamster sentinel system was therefore established at

Figure 7. Bleeding a sentinel hamster using the cardiac puncture technique.

Figure 8. Bleeding a sentinel hog from its vena cava.

the Welder Refuge in an attempt to detect VEE activity during the late spring and summer of 1972 and 1973. Sentinel sites transected the Refuge and represented possible "VEE mosquito" areas (Figure 9). One hamster was placed in each of four ½-inch mesh wire cages measuring approximately 1 ft. x 1 ft. x 1 ft. The cages were hung from trees approximately 4 feet off the ground. (This part of the sentinel system was deleted in 1973 due to its questionable value.) At each of three other ground stations, three hamsters were placed in a large wire cage situated approximately 6 inches off the ground (Figure 10). Hamsters of various colors and sex were utilized at each of the sites. Blood samples were taken every two weeks from hamsters located in the four tree sites. Blood samples were taken weekly from a hamster at each of the three ground stations. Any hamster which died during the study was frozen and virus isolations were attempted.

A domestic hog sentinel system was established in the spring of 1973 to compliment the hamster sentinel system because of the large number of feral hog reactors to VEE found on the Refuge in 1972. Three sites, one in close proximity to each of the three hamster sites, were selected. (Figure 9). One of the two pigs at each site was bled every week and the serum tested. Pens were made of a large mesh wire of at least 1 inch square and were approx-

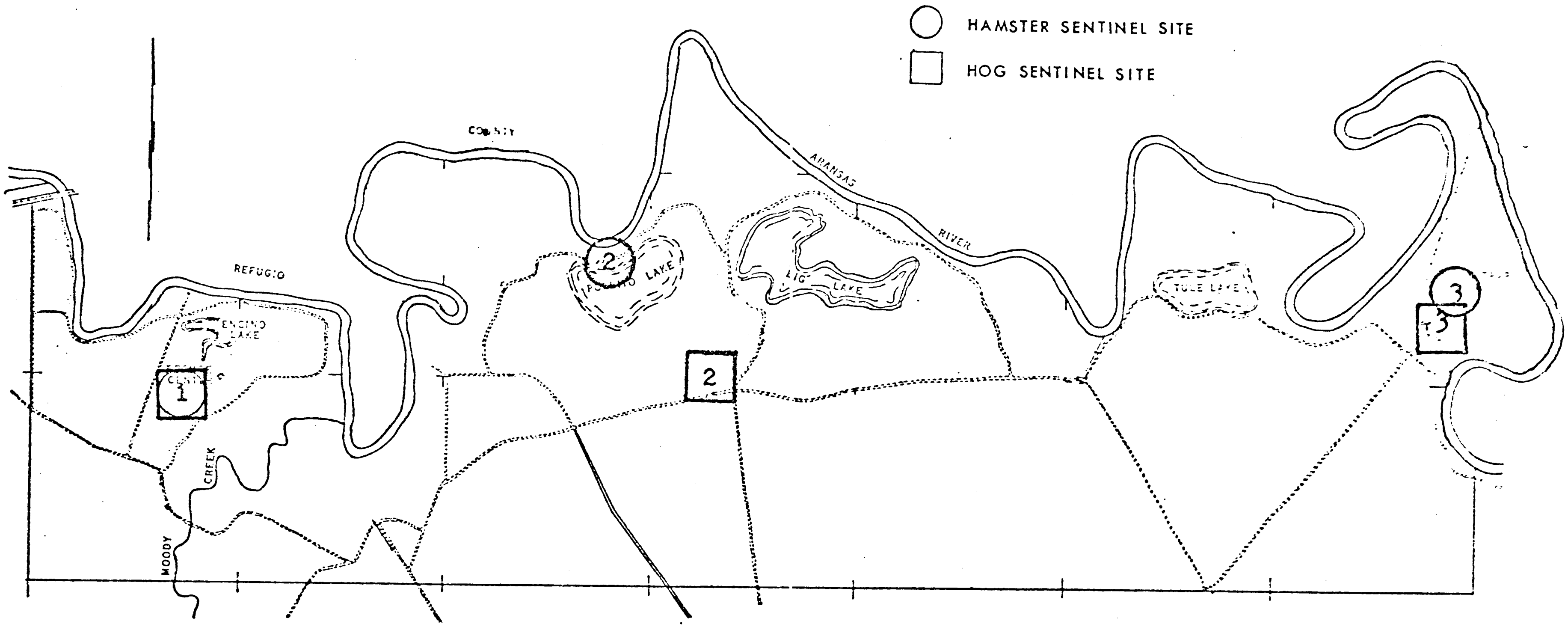

Figure 9. Location of hamster and pig sentinel sites on the Welder Wildlife Refuge.

Figure 10. Sentinel hamsters and cage located at site No. 2.

imately 10 feet in width and 20 feet in length (Figure 11). Pigs were allowed to roam freely within these pens.

Laboratory Testing

Serum samples were heat-inactivated at 56°C for 30 minutes and tested for the presence of neutralizing antibody to VEE and Western equine encephalitis (WEE) in a HeLa cell metabolic inhibition test (Kissling and Kuns, 1958). Virus stocks were prepared by infecting two-to-five day old mice with approximately 1000 LD50 of seed virus. A 10 percent suspension was prepared by homogenizing the brain tissue of infected mice in diluent consisting of mixture 199 (a 10X stock solution) with 20 percent calf serum, 1000 units of penicillin and 500 micrograms of streptomycin per ml. After centrifugation at 2000 revolutions per minute for 10 minutes, the supernatant fluid was dispensed in vials and stored in a mechanical freezer held at -65°C. The identity of all virus preparations was confirmed by neutralization tests with antisera of known specificity. The Fleming isolate was used as the WEE viral stock. TC-80 was the VEE viral strain used in the tests.

All viral assays were done using a standard plaque test in a HeLa cell line (Thomas and Trainer, 1970), or in two-to-four day old suckling mice inoculated intracerebrally. Five to eight mice were used per sample and observed for

Figure 11. Sentinel hogs and their pen located at site No. 3.

clinical signs daily for 14 days. Animals with severe signs were sacrificed and pooled with mice found soon after death. A suspension was made of the brain tissue taken from these animals and inoculated into suckling mice. This procedure was repeated until identity of the causative agent of death was confirmed by neutralization tests with antisera of known specificity. Blood clots from animal blood samples taken in 1972 were dispersed by aspiration into and from a tuberculin syringe and diluted 1:5 in Hank's balanced salt solution. The red blood cell and plasma fractions were treated by sonification with a Branson sonifer prior to diluting (Hoff and Trainer, 1972). Spleen and brain tissue from hamsters, mosquitoes, and other virus suspected specimens were emulsified as a 10 percent suspension in diluent consisting of mixture 199 with 20 percent calf serum, 1000 units of penicillin and 500 micrograms of streptomycin per milliliter and inoculated into suckling mice. Swab eluates were tested in mice as 1:2 dilutions.

Serologic tests for WEE were undertaken to help determine the possibility of cross reaction between WEE and VEE neutralizing antibody.

RESULTS

More than 2,600 serum samples from a variety of reptiles, birds, and mammals were tested for VEE and WEE reactors. These sera were collected during a 10 year period, beginning in 1963; sera collected prior to 1972 were available from previous studies (Cook et al. 1965; Trainer and Hanson, 1969; Trainer, 1970). Sera from 1972 and 1973 were collected as part of this investigation.

A total of 7565 trap nights were expended to capture animals for sera collection in 1972 and 1973. Over-all trapping success was approximately 5 percent (Table 1). Trap success of the various sized live traps can be used as a relative estimate of the small mammal population densities on the Refuge. The increase in large-size live trap success in 1973 was a reflection of an apparent eruption of the raccoon population. A change in the location of trap lines was responsible for the increase in medium-size trap success. Wood rats (Neotoma micropus) were almost exclusively captured in the medium-size Hav-a-hart traps.

A variety of wild and domestic species on the Welder Refuge were VEE reactors prior to 1971 (Table 2), including deer (Odocoileus virginianus), cattle (Bos taurus), rabbits (Sylvilagus floridanus), goats (Capra hircus), feral hogs (Sus scrofa), peccaries (Pecari angulatus), raccoon

Table 1. Summary of the number of trap nights and their capture success during 1972 and 1973 at the Welder Refuge.

Year	Trap Size*			Total
	Large	Medium	Small	
1972	161 (3)	120 (0)	4125 (5)	4406 (5)
1973	520 (9)	313 (4)	2263 (4)	3159 (5)
Total	681 (7)	433 (3)	6388 (5)	7565 (5)

*Large (32"x 11"x 13") and medium traps (18"x 5¼"x 5¼") were Hav-a-hart type; small traps (10"x 3"x 3¼") were Sherman type live traps. ( ) = percent trap success.

Table 2. Summary of VEE serologic results for selected mammal populations at the Welder Wildlife Refuge.

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)	...
Armadilo 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 megalotis	...	...	0/1 (0)	0/5 (0)
White-footed Mouse Peromyscus leucopus	...	...	0/3 (0)	0/7 (0)
House Mouse Mus musculus	...	...	...	0/1 (0)

*Number reactors/number tested; ( ) = percent reactors; ... = none tested.

(*Procyon lotor*), and pocket gophers (*Geomys bursarius*).

Of particular interest were serologic results for feral hog and white-tailed deer populations. Feral hog populations had high reactor rates of 43, 59, and 75 percent during the study period (Table 2).

VEE reactors were detected in deer every year of the study with the highest reaction rates occurring in 1967, 1969, 1971, and 1973 (Table 3). VEE reactors were compared with age and sex of the deer utilizing the chi-square test. The prevalence of VEE reactor rates increased with age (Table 4). The number of deer reactors two years and older was significantly greater (99 percent level) than the number of fawn reactors. The number of female deer reactors was significantly larger (90 percent level) than the number of male reactors (Table 5). Reaction rates due to sex differences occurred at the 95 percent level of significance among deer collected prior to 1971. Reaction rates in deer collected after 1971 showed no significant difference due to sex. There was no detectable difference in reaction rates associated with sex in the individual age categories (Table 6).

There was little or no serologic activity detected in more than 250 rodents, 75 raccoons, 30 opossum, and 126 coyotes, yet these species are reported to be involved in VEE epizootiology in other studies.

More than 1000 turkey (*Meleagris gallopavo*) sera were

Table 3. Summary of serologic results of white-tailed deer at the Welder Wildlife Refuge to VEE and WEE (1963-1973).

Year	VEE*	WEE*
1963	3/24 (12)	0/24 (0)
1964	11/39 (27)	10/39 (26)
1965	19/80 (24)	15/80 (19)
1966	1/13 (7)	1/13 (7)
1967	26/55 (47)	15/55 (27)
1968	10/36 (28)	2/36 (5)
1969	13/35 (37)	2/35 (6)
1970	9/62 (15)	5/62 (8)
1971	29/70 (41)	12/70 (17)
1972	13/50 (26)	4/30 (13)
1973	16/45 (36)	6/35 (17)

*Number reactions/number tested; ( ) = percent tested

Table 4. Comparison of white-tailed deer VEE reactors by age.

Year	Age in Months 0-12	13-24	25-36	37-48	49+	Total	Chi Square Value
Pre-1971	3/55* (5)	5/29 (17)	7/22 (32)	6/17 (35)	8/25 (32)	29/148 (32)	< .01[a]
1971	3/9 (33)	6/10 (60)	3/9 (33)	3/11 (27)	6/16 (38)	21/55 (38)	
Post-1971	3/14 (21)	2/15 (13)	15/46 (33)	6/15 (40)	7/21 (33)	23/80 (29)	< .1[b]
Total	9/78 (12)	13/54 (24)	15/46 (33)	15/43 (35)	21/62 (34)		< .01[c]

*Number of reactors/number tested; ( ) = percent reactors.

[a]Total pre-1971 reactors compared to total 1971 reactors.

[b]Total post-1971 reactors compared to total 1971 reactors.

[c]Reactors of 0-12 months of age compared to reactors of 25 or more months of age.

Table 5. Comparison of white-tailed deer VEE reactors by sex according to year of collection.

Year	Male	Female	Chi Square Value
Pre-1971	7/63 (11)*	22/85 (26)	< 0.05
1971	5/20 (25)	14/35 (40)	< 0.1
Post-1971	10/34 (29)	13/46 (28)	0.0
Total	22/117 (19)	49/160 (30)	< 0.1

*Number of reactions/number tested; ( ) = percent reactors.

Table 6. Comparison of white-tailed deer VEE reactors by sex according to age.

Age*	0 - 12		13 - 24		25 - 36		37 - 48		49 +	
Year	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Prior to 1971	1/31‡ (3)	2/24 (8)	2/11 (18)	3/18 (17)	3/9 (33)	4/13 (31)	0/5 (0)	6/12 (50)	1/7 (14)	7/18 (39)
1971	0/2 (0)	3/7 (43)	1/5 (20)	3/5 (60)	1/3 (33)	2/6 (33)	0/1 (0)	3/10 (30)	3/9 (33)	3/7 (43)
Post-1971	2/8 (25)	1/6 (17)	0/4 (0)	2/11 (18)	3/10 (30)	2/5 (40)	3/4 (75)	3/11 (27)	2/8 (25)	5/13 (38)
Total	3/41 (7)	6/37 (16)	3/20 (15)	8/32 (25)	7/22 (32)	8/24 (33)	3/10 (30)	12/33 (36)	6/24 (25)	15/38 (39)
Chi sq. Value	$< .2$		$< .2$		$< .5$		$< .5$		$< .2$	

*Age given in months.

‡Number of reactors/number tested; ( ) = percent reactors.

examined for VEE antibody; less than 2 percent were reactors. None of the 87 black and turkey vultures (fam. Cathartidae) tested were reactors (Table 7). Although only limited numbers of reptile species were tested, there were few, if any, VEE reactors in these populations (Table 7).

The possibility of cross reactions between two group A arboviruses and/or dual infection in the specimens tested was considered and all sera were tested for WEE and VEE (Table 8). Serologic results of cattle, goat, and peccary sera indicate the possibility of cross reactions between WEE and VEE; while those of feral hogs, turkey, and deer sera suggest that dual infections may be occurring. There were few, if any, cross reactions occurring for other species.

There was no serologic or virologic evidence of VEE activity detected during 1972 and 1973 in the hamster sentinel system. Although several hamster deaths occurred at a time when mosquito populations were at their peak, no virus was isolated and their cause of death was unknown. Serologic results of the domestic hog sentinels on the Refuge were confused by the fact that low levels of neutralization antibody for VEE were detected in sera taken prior to exposure of the sentinels on the Refuge.

Table 7. Summary of VEE serologic results for selected avian and reptilian populations at the Welder Wildlife Refuge.

Host	Prior to 1971	1971	1972	1973
Turkey *Meleagris gallopavo*	16/993 (2)*	0/29 (0)	0/33 (0)	0/24 (0)
Vulture fam. Cathartidae	0/3 (0)	...	0/39 (0)	0/45 (0)
Rattlesnake *Crotalus atrox*	1/7 (13)	...	...	...
Bull Snake *Pituophis catenifer*	...	...	0/2 (0)	...
King Snake *Lampropeltis getulus*	...	...	0/2 (0)	...
Rat Snake *Elaphe obsoleta*	...	...	0/2 (0)	...
Alligator *Alligator mississipiensis*	...	...	...	0/2 (0)
Turtle	0/3 (0)	...	...	...

*Number of reactors/number tested; ( ) = percent reactors; ... = none tested.

Table 8. VEE reactors showing possible cross reactivity with WEE.

Host	VEE	WEE	VEE & WEE	Total
Cattle *Bos taurus*	5	20	63	89/167*
Goat *Capra hircus*	0	0	2	2/4
Deer *Odocoileus virginianus*	104	40	29	173/406
Peccary *Pecari angulatus*	4	0	8	12/30
Feral Hog *Sus scrofa*	29	3	2	34/51
Turkey *Meleagris gallopavo*	9	85	8	102/1076

*Number of reactors/number tested.

Vector Study

Six genera of mosquitoes including *Psorophora* spp. and *Culex* spp. were collected on the Welder Refuge at various times during 1972 and 1973 (Table 9). In 1972 and 1973, 97 and 60 percent of the mosquitoes, respectively, were in the genus *Psorophora*; three and 35 percent to the genus *Culex* (Table 10). Certain genera were found only at specific sites. For example, *Culex* mosquitoes were never collected at site number 1 and *Psorophora* comprised over 90 percent of the mosquitoes collected at this site. *Culex* mosquitoes comprised 60 and 53 percent of the specimens collected at sites 2 and 3, respectively, with the remainder consisting mostly of *Psorophora*.

Examination of permanent and temporary bodies of water revealed that *Psorophora* mosquito larvae inhabited temporary shallow pools and puddles while *Culex* and *Aedes* larvae were detected in larger, more permanent bodies of water.

A comparison is presented in Figure 12 between the prevalence of mosquitoes and rainfall. A peak population of *Psorophora* was reached approximately one week after the first heavy rain (3 inches or more). As the heavy rain continued, the *Psorophora* population declined and *Culex* mosquitoes increased. This increase continued until the the study was terminated in August 1973.

A comparison between spring rainfall and the percen-

Table 9. Mosquito collections at the Welder Wildlife Refuge (1972-1973).

Date	*Mansonia*	*Aedes*	*Culex*	*Psorophora*	*Culiseta*	*Anopheles*	Total
5 July 72	0	0	0	0	0	0	0
14 July 72	0	0	2 (100)*	0	0	0	2
21 July 72	0	0	0	0	0	0	0
3 Aug. 72	0	0	0	1 (100)	0	0	1
9 Aug. 72	0	0	0	64 (100)	0	0	64
1 Jan. 73	0	0	0	0	15 (83)	3 (17)	18
8 June 73	0	0	0	2 (22)	7 (78)	0	9
16 June 73	3 (6)	1 (2)	0	50 (92)	0	0	54
22 June 73	0	0	0	87 (100)	0	0	87
29 June 73	0	2 (2)	6 (7)	82 (91)	0	0	90
5 July 73	0	0	7 (78)	2 (22)	0	0	9
12 July 73	0	0	26 (100)	0	0	0	26
19 July 73	0	0	20 (100)	0	0	0	20
26 July 73	0	0	26 (90)	0	0	3 (10)	29
2 Aug. 73	0	0	46 (98)	0	0	1 (2)	47
Total	3 (1)	3 (1)	133 (29)	298 (65)	22 (5)	7 (1)	456

*( ) = percent composition of total mosquitoes captured; collection period was 20 minutes per site.

Table 10. Mosquito genera (adults) collected at the three hamster sentinel sites on the Welder Wildlife Refuge (1973).

Genera	Site #1	Site #2	Site #3	Total
Mansonia	3 (2)*	0 (0)	0 (0)	3 (1)
Aedes	2 (1)	1 (1)	2 (2)	5 (1)
Culex	0 (0)	88 (60)	43 (53)	131 (35)
Psorophora	131 (92)	56 (39)	36 (43)	223 (60)
Culiseta	7 (5)	0 (0)	0 (0)	7 (2)
Anopheles	0 (0)	1 (1)	2 (2)	3 (1)
Total	143	146	100	

*( ) = percent composition of total mosquitoes captured; mosquitoes collected at each site for a total of 280 minutes.

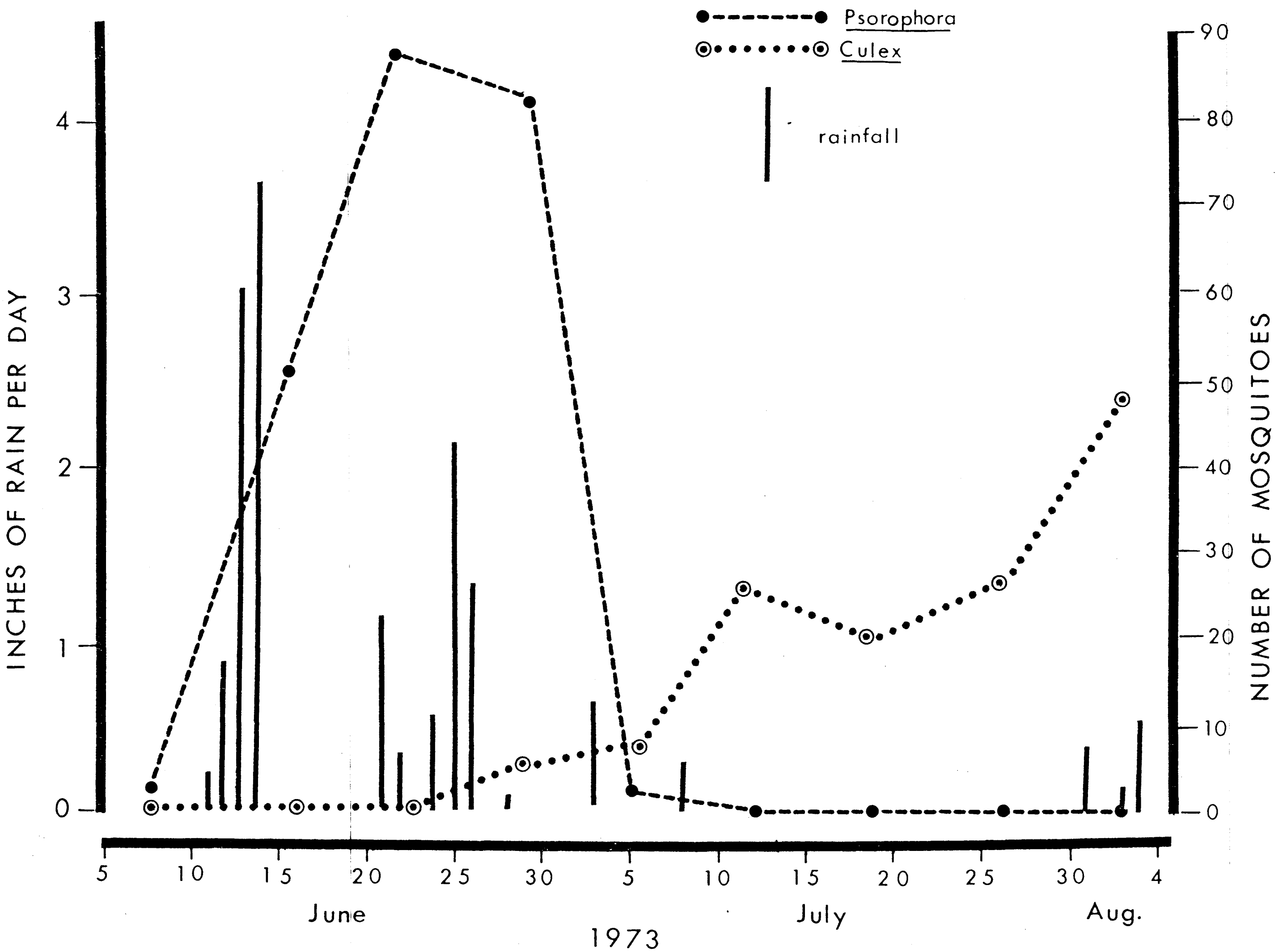

Figure 12. Comparison of rainfall and the abundance of Culex and Psorophora mosquitoes.

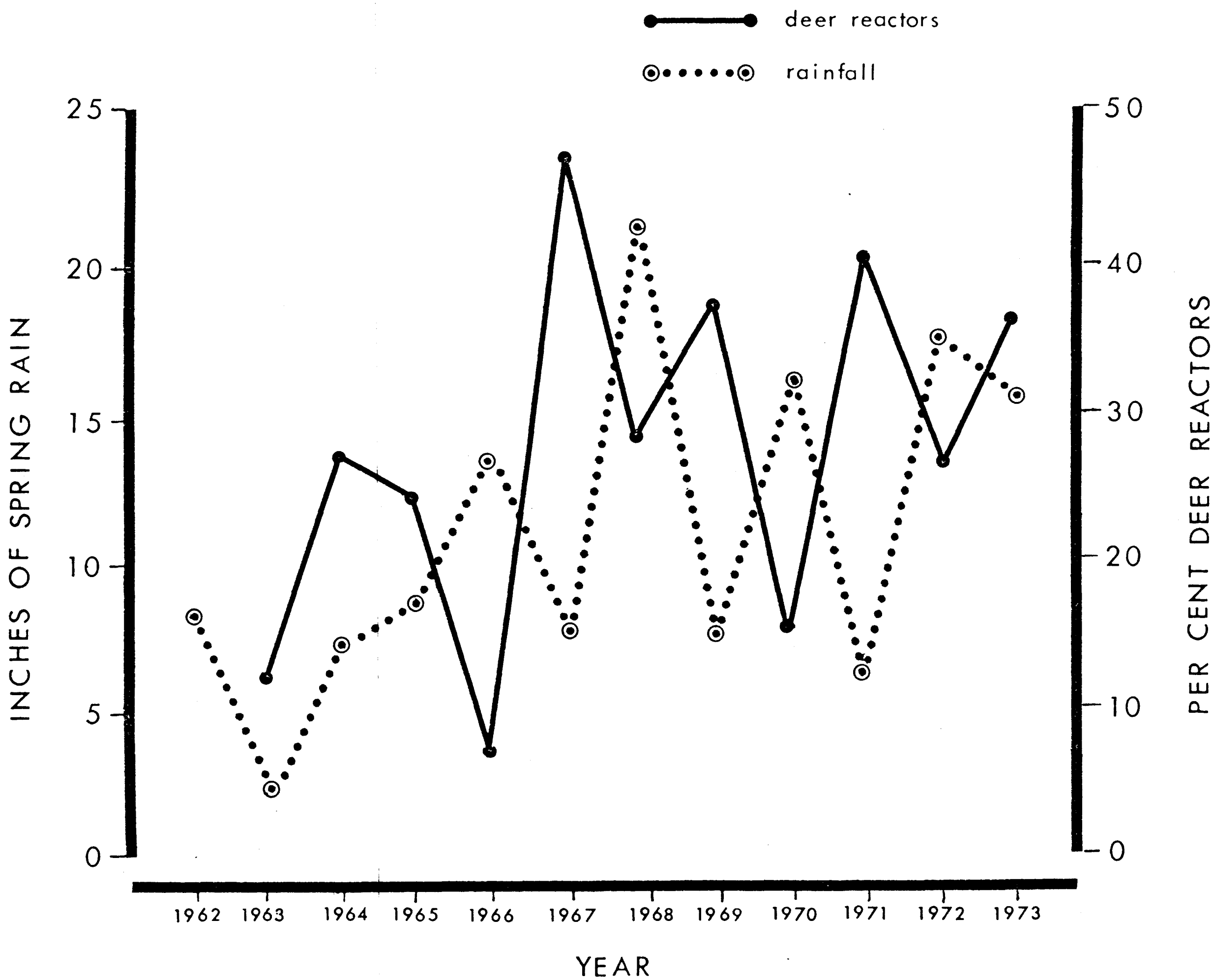

Figure 13. Comparison of spring rainfall and the abundance of deer VEE reactors.

tage of white-tailed deer reactors resulted in some interesting findings. Heavy spring rainfall appeared to be followed by high deer reactor rates the following year (Figure 13).

Rainfall data on the Welder Refuge was compiled by W. C. Glazener (Table 11).

Table 11. Rainfall at the Welder Wildlife Refuge (1962-1973).*

Year	Winter	Spring	Summer	Fall	Total
1962	1.20	7.07	5.57	4.67	18.51
1963	1.49	2.24	5.17	7.85	16.75
1964	5.31	7.00	15.73	8.19	36.23
1965	4.34	8.78	4.05	10.70	27.87
1966	5.40	13.83	8.32	4.47	32.02
1967	4.49	7.71	25.52	4.96	44.05
1968	5.96	20.87	10.56	10.87	48.17
1969	6.86	7.65	7.65	12.50	34.66
1970	6.38	16.12	13.43	4.50	40.43
1971	0.51	6.28	25.08	7.27	39.14
1972	4.27	17.54	14.39	3.10	39.30
1973	5.57	15.48	18.87	...	...
Average	4.25	10.88	12.86	7.18	34.28

*Rainfall data compiled by W. C. Glazener, Assistant Director of the Welder Wildlife Refuge; rainfall recorded in inches.

DISCUSSION

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 (Cook et al. 1965). In addition, experimental studies on selected animal species such as deer have been done and expected antibody responses have been varified (Hoff and Trainer, 1972). WEE was also tested to examine the specificity of VEE reactors. Specific reactions occurred in most instances but cross reactions may have occurred in cattle, goat, and peccary sera.

A number of different wild and domestic populations were screened serologically with the MIT to detect evidence of VEE activity at the Welder Wildlife Foundation prior to, during, and after, the 1971 epizootic. Based on these serologic results, a number of species had experience with VEE prior to 1971 including cattle, gophers, deer, goats, peccaries, raccoons, feral hogs, rabbits, and coyotes. These findings and the results of Cook et al. (1965) suggest that VEE was present and possibly enzootic in Texas prior to the 1971 epizootic; a situation which may be similar to that reported in Florida (Bigler, 1971).

Scherer et al. (1971) 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 suggest a similar situation, although cross reactions may have occurred in cattle sera making these VEE results questionable.

In the deer population, the VEE reactor rate fluctuated each year and an biennial pattern appeared to occur. This biennial rhythm may have been due to an increase in susceptible white-tailed deer numbers every other year and to the prevalence of mosquitoe vectors which followed a biennial pattern and were determined by the amount of spring and summer rainfall.

High spring rainfall was recorded every year prior to a peak year for deer VEE reactors (Figure 13). Low spring rainfall occurred during the peak years. Mosquito data collected during this study suggest that when a large amount of rain (over 3 inches) occurs, mosquito populations increase. The first genus of mosquito to react to rainfall is Psorophora. This mosquito was found regularly and in great numbers in shallow temporary pools and puddles formed from initial heavy rainfall. In 1973, the Psorophora population reached a peak approximately one week after the initial rainfall, then declined rapidly even though the rains continued; a similar response was recorded in 1972. The Culex mosquito population increased gradually after the accumulated rainfall raised the water level of the permanent bodies of water. This rise in the water level evidently afforded greater protection against predators for mosquito larvae which inhabited these waters and increased the area in which they may breed. Since *Culex* mosquitoes, whose larvae inhabit permanent bodies of water, are one of the most important vectors of enzootic VEE, a large population of these mosquitoes would theoretically increase virus transmission opportunities. A marked seasonal variation in the amount of rainfall occurs at the Welder Wildlife Refuge, with most rain occurring during the spring and summer. Thus, *Culex* populations may not develop until relatively late in the year because of the lag in population growth after a rainfall. The result might be that VEE was transmitted late in the year; thus explaining the lag in the number of deer reactors to VEE, since most deer were collected during the winter and spring months and recorded as collected in that year.

A high percentage of adult deer, especially females, were reactors to VEE. Maternal antibody from these females may also delay VEE infections in young fawns. Levels of maternal antibody in fawns decrease after several months, making them susceptible to VEE infection at approximately the same time that the *Culex* mosquito population reaches its peak.

Reactor rates increased with age. The number of fawns that were reactors to VEE was significantly smaller (99 percent level) than the number of deer reactors over two years of age. Older deer may have a greater opportunity for VEE exposure; maternal immunity may also play a role as discussed earlier. Since deer collections were made primarily during winter and spring months, very little sera from fawns (1-3 months) were tested. Most of the fawn sera tested were from fawns 6-12 months of age and maternal antibody would not be detectable.

A high percentage of the feral hogs tested prior to 1971 and in 1972 and 1973 were VEE reactors. These results, along with results of experimental studies in hogs (Dickerman et al. 1972) suggest that feral hogs may serve as an indicator host 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 (Grayson and Galindo, 1968; Jonkers et al. 1968; Shope and Woodall, 1973). Results of this study do not incriminate rodents as significant species in the long-term maintenance of VEE in South Texas since none of the more than 250 rodents tested in 1972 and 1973 were reactors.

Other studies have suggested that the opossum (<u>Didelphis</u>

marsupialis)(USDA, 1973), rabbit (Hess, 1972), coyote (Lundgren and Smart, 1969), and vulture (Grayson and Galindo, 1968) may have been involved in recent epizootics of the IB subtype of VEE. The opossum and the raccoon were also incriminated as possible disseminating hosts of VEE virus in Florida (Bigler, 1971; Lord et al. 1973). Results of this serologic study would not incriminate these species in the epizootiology of VEE in South Texas.

Hess (1972) reported a high VEE reactor rate in rabbits from Texas and Mackenzie (1972) 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. In 1972, rabbit populations on the Welder Refuge were extremely low and may have been a result of mortality during the 1971 epizootic.

Recognizing the limitations of this vector study, such as the use of a human as an attractant for mosquitoes and the susceptibility of mosquitoes to aspiration, the prevalence of mosquitoes on the Welder Refuge was not totally evaluated. The most numerous species of mosquito on the Refuge belonged to the genera Psorophora and Culex. A very small percentage of total mosquitoes collected belonged to other genera.

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 involved. Isolation of VEE virus from wild populations is essential to confirm VEE activity in this region. The yearly collection of deer on the Welder Refuge may serve as a good opportunity to monitor VEE activity in South Texas. Several other animal populations such as the rabbit and peccary populations are "suspicious" and should be studied in greater detail.

LITERATURE CITED

Andrews, C. H. and H. G. Pereira. 1967. Viruses of the Vertebrates. Williams and Wilkins Co., Baltimore, Maryland. 425 pp.

Bigler, W. J. 1971. Serologic evidence of Venezuelan equine encephalitis virus infections in raccoons of south central Florida. J. Wildl. Dis. 7: 166-170.

Box, T. W. and A. D. Chamrad. 1966. Plant communities of the Welder Wildlife Refuge. Contrib. B-5, Welder Wildlife Foundation, Sinton, Texas.

Carpenter, S. J. and W. J. LaCasse. 1955. Mosquitoes of North America. Univ. of California Press, Berkeley. 360 pp.

Chamberlain, R. W., R. E. Kissling, D. D. Stamm, D. B. Nelson, and R. K. Sikes. 1956. Venezuelan equine encephalomyelitis in wild birds. Amer. J. Hyg. 63(3): 261-273.

Cook, R. S., D. O. Trainer, W. C. Glazener, and B. D. Nassif. 1965. A serologic study of infectious diseases of wild populations in South Texas. Trans. 30th N. Amer. Wildl. Nat. Res. Conf. 142-155.

Davis, W. B. 1960. The Mammals of Texas. Texas Game and Fish Commission, Austin, Texas. 252 pp.

Dickerman, R. W., G. J. Baker, J. V. Ordonez, and W. F. Scherer. 1973. Venezuelan equine encephalomyelitis viremia and antibody responses of pigs and cattle. Amer. J. Vet. Res. 34(3): 357-361.

Galindo, P. 1972. Endemic vectors of Venezuelan encephalitis. In Venezuelan Encephalitis. Proc. Workshop-Symposium on Venezuelan encephalitis. P.A.H.O. Sci. Pub. 243: 249-253.

Gilyaad, R. T. 1945. A clinical study of Venezuelan equine encephalitis virus in Trinidad, B. W. I. J. Amer. Vet. Med. Assoc. 106: 267-277.

Glazener, W. C. 1973. Personal communication. Assistant Director, Welder Wildlife Foundation, Sinton, Texas.

Gonzalez, A. M. and J. M. Macias. 1969. Estudio de una epizootia de encefalitis equna de Venezuela occurrida en Tampaulipas, Mexico. Rev. Invest. Salud. Publica. 29(1): 3-20.

Grayson, Margaret A. and P. Galindo. 1968. Epidemiologic studies of Venezuelan equine encephalitis virus in Almirante, Panama. Amer. J. Epidemiol. 88(1): 80-96.

Grayson, Margaret A. and P. Galindo. 1969. Ecology of Venezuelan equine encephalitis virus in Panama. J. Amer. Vet. Med. Assoc. 155(12): 2141-2145.

Grimes, J. E. 1972. Serological responcse of horses vaccinated with the TC-83 live attenuated Venezuelan equine encephalomyelitis virus. Southwestern Vet. 25(2): 125-129.

Groot, H., J. A. Kerr, C. San-Martin, and H. Vidales. 1959. Antibodies to yellow fever and other arthropod-borne viruses in human residents of San Vincente de Chucuri Santander, Colombia. Amer. J. Trop. Med. 8: 175-189.

Gutierrez, E. 1972. Venezuelan encephalitis in Ecuâdor. In Venezuelan Encephalitis. Proc. Workshop-Symposium on Venezuelan Encephalitis. P.A.H.O. Sci. Pub. 243: 195-197.

Hess, A. 1972. Discussion: The role of silent vertebrate hosts. In Venezuelan Encephalitis. Proc. Workshop-Symposium on Venezuelan Encephalitis. P.A.H.O. Sci. Pub. 243: 247-248.

Hoff, G. L. and D. O. Trainer. 1972. Experimental infection of Venezuelan encephalomyelitis virus in white-tailed deer. Amer. J. Epidemiol. 96: 379-382.

Hoff, G. L., T. M. Yuill, J. O. Iverson, et al. 1970. Selected microbial agents in snowshoe hares and other vertebrates of Alberta. J. Wildl. Dis. 6: 472-478.

Hubert, A. H. 1973. Checklist of arthropods collected on the Welder Wildlife Refuge. Academy of Health Sci., U. S. Army, Fort Sam Houston, Texas.

Jones, F. B., C. M. Rowell, and M. C. Johnston. 1961. Flowering plants and ferns of the Texas Coastal Bend Counties. Contrib. B-5, Welder Wildlife Foundation, Sinton, Texas.

Jonkers, A. H., L. Spence, W. G. Downs, T. H. G. Aitken, and C. B. Worth. 1968. Arbovirus studies in Bush Bush Forest, Trinidad, W. I., September 1959-December 1964. VI. Rodent-associated viruses. Amer. J. Trop. Med. Hyg. 17(2): 285-298.

Kie, J. G. 1973. Personal communication. School of Forestry and Conservation. Univ. of California, Berkeley, California.

Kissling, R. E., R. W. Chamberlain, D. B. Nelson, and D. D. Stamm. 1956. Venezuelan equine encephalomyelitis in horses. Amer. J. Hyg. 63(3): 274-287.

Kissling, R. L. and M. L. Kuns. 1958. A colorimetric neutralization test for arthropod-borne animal viruses. Public Health Service, Virus and Rickettsial Section, Center for Disease Control, Montgomery, Alabama.

Kubes, V. and F. A. Rios. 1939. The causative agent of infectious equine encephalomyelitis in Venezuela. Science. 90: 20-21.

Lord, R. D., C. H. Calisher, W. D. Sudia, and T. H. Work. 1973. Ecological investigations of vertebrate hosts of Venezuelan equine encephalomyelitis virus in South Florida. Amer. J. Trop. Med. Hyg. 22(1): 116-123.

Lundgren, D. L. and K. L. Smart. 1969. Experimental infection of coyote pups with Venezuelan equine encephalomyelitis virus. Amer. J. Trop. Med. Hyg. 18(2): 268-272.

Madalengiutia, J., O. Palacios, J. C. Ubiliuz, and S. Alua. 1972. An outbreak of Venezuelan encephalitis virus in man in the Tumbes Department of Peru. In Venezuelan Encephalitis. Proc. Workshop-Symposium on Venezuelan Encephalitis. P.A.H.O. Sci. Pub. 243: 195-197.

Michael, E. D. 1965. Movements of white-tailed deer on the Welder Wildlife Refuge. J. Wildl. Mgmt. 29: 44-52.

Monlux, W. S. and A. J. Luedke. 1973. Brain and spinal cord lesions in horses inoculated with Venezuelan equine encephalomyelitis virus. Amer. J. Vet. Res. 34(4): 465-473.

Nichols, J. B. and W. J. Bigler. 1967. Animal sentinels in a statewide arthropod borne encephalitis surveillance program. J. Amer. Vet. Med. Assoc. 151(12): 1767-1771.

Omohundro, R. E. 1972. Venezuelan equine encephalomyelitis emergency operation. J. Amer. Vet. Med. Assoc. 161(11): 1516-1518.

Otteni, L. C. 1971. Predator-prey relationships and reproduction of the barn owl in Southern Texas. M. S. Thesis. Texas Technical Univ., Lubbock, Texas.

Parker, R. L., P. B. Dean, and R. B. Zehmer. 1973. Public health aspects of Venezuelan equine encephalitis. J. Amer. Vet. Med. Assoc. 162(9): 777-779.

Pennak, R. W. 1953. Freshwater Invertebrates of the United States. Ronald Press, New York, New York. 769 pp.

Sanmartin-Barberia, C., H. Groot and E. Osorno-Mesa. 1954. Human epidemic in Columbia caused by the Venezuelan equine encephalitis virus. Amer. J. Trop. Med. 3: 283-293.

Scherer, W. F., R. W. Dickerman, C. Campillo-Sainz, M. L. Zarate, and F. Gonzales. 1971. Ecologic studies of Venezuelan encephalitis virus in southeastern Mexico. V. Infection of domestic animals other than equines. Amer. J. Trop. Med. Hyg. 20(6): 989-993.

Scherer, W. F., R. W. Dickerman, R. R. LaFiandra, C. Wong Chia, and J. Terrian. 1971. Ecologic studies of Venezuelan encephalitis virus in southeastern Mexico. VI. Infections of wild mammals. Amer. J. Trop. Med. Hyg. 20(6): 980-988.

Scherer, W. F., R. W. Dickerman, and J. V. Ordonez. 1970. Discovery and geopraphic distribution of Venezuelan encephalitis virus in Guatemala, Honduras, and British Honduras during 1965-68, and its possible movement to Central America and Mexico. Amer. J. Trop. Med. Hyg. 19(4): 703-711.

Scherer, W. F., R. W. Dickerman, C. Wong Chia, A. Ventura, A. Moorhouse, R. Geiger, and A. Diaz Najera. 1964. Venezuelan encephalitis virus in Veracruz, Mexico, and the use of hamsters as sentinels. Science 145: 274-275.

Scherer, W. F., J. V. Ordonez, et al. 1972. Observations of equines, humans, and domestic and wild vertebrates during the 1969 equine epizootic and epidemic of Venezuelan encephalitis in Guatemala. Amer. J. Epidemiol. 95(3): 255-266.

Shope, R. E. and J. P. Woodall. 1973. Ecological interaction of wildlife, man, and a virus of the Venezuelan equine encephalomyelitis complex in a tropical forest. J. Wildl. Dis. 9: 198-203.

Spertzel, R. O. 1971. Overview of the 1971 Texas VEE epizootic. Proc. 75th Annual Meeting U. S. Animal Health Assoc. 162-165.

Spertzel, R. O. and R. W. McKinney. 1970. VEE, A disease on the move. Proc. 74th Annual Meeting U. S. Animal Health Assoc. 268-275.

Srihongse, S., W. F. Scherer, and P. Galindo. 1967. Detection of arboviruses by sentinel hamsters during the low period of transmission. Amer. J. Trop. Med. Hyg. 16(4): 512-523.

Sudia, W. D. 1972. Arthropod vectors of epidemic Venezuelan equine encephalitis. In Venezuelan Encephalitis. Proc. Workshop-Symposium on Venezuelan Encephalitis. P.A.H.O. Sci. Pub. 243: 157-161.

Sudia, W. D. and V. F. Newhouse. 1971. Venezuelan equine encephalitis in Texas, 1971 informational report. Mos. News 31(3): 350-351.

Thomas, F. C. and D. O. Trainer. 1970. Bluetongue virus. (1) In pregnant white-tailed deer. (2) A plaque reduction neutralization test. J. Wildl. Dis. 6: 384-389.

Thorpe, B. D., K. L. Smart, and R. W. Sidwell. 1965. Arbovirus complement fixing antibodies in sera of wildlife of West Central Utah. Soc. Exp. Bio. Med. Proc. 118(1): 179-181.

Trainer, D. O. 1973. Wildlife as monitors of disease. Amer. J. Public Health.63(3): 201-204.

Trainer, D. O. 1970. The use of wildlife to monitor zoonoses. J. Wildl. Dis. 6: 397-401.

Trainer, D. O. and R. P. Hansen. 1969. Serologic evidence of arbovirus infections in wild ruminants. Amer. J. Epidemiol. 90(4): 354-358.

United States Army. 1972. Medical Entomology Handbook. Medical Field Service School, Brooke Army Medical Center, Ft. Sam Houston, Texas.

United States Department of Agriculture. 1973. The Origin and Spread of Venezuelan Equine Encephalomyelitis. Animal and Plant Health Inspection Service, APHIS 91-10, 51pp.

United States Department of Agriculture. 1972. Venezuelan Equine Encephalomyelitis Surveillance Program 1972. Veterinary Services, APHIS, Foreign Animal Disease Report 1. 8pp.

Walton, T. E., F. E. Brautigan, J. A. Ferrer, and K. M. Johnson. 1972. Epizootic Venezuelan equine encephalomyelitis in Central America - Disease pattern and vaccine evalution in Nicaragua, 1969-1970. Amer. J. Epidemiol. 93(3): 247-254.

White, M. 1967. Population ecology of some whitetail deer in South Texas. Ph. D. Thesis. Purdue Univ., Lafayette, Indiana. 214pp.

Young, N. A. 1972. Origin of epidemics of Venezuelan equine encephalitis. J. Inf. Dis. 125(5): 565-567.

Young, N. A. and K. M. Johnson. 1969. Antigenic variants of Venezuelan equine encephalitis virus: Their geographic distribution and epidemiologic significance. Amer. J. Epidemiol. 89: 286-307.