

Epidemiological Aspects of Venezuelan Equine Encephalitis Virus Infections

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INTRODUCTION

The modern history of man has been often marked with outbreaks of virus infections. Noted among these outbreaks are those caused by viruses known to be transmitted by arthropod vectors and hence classified as arthropod-borne (arbo) viruses. One of the least investigated of the arboviruses, although potentially a serious disease threat to man, is the virus causing Venezuelan equine encephalitis (VEE). VEE virus has been thought to be limited primarily to certain segments of South and Central America and Trinidad, but evidence of this disease agent has recently also been found in portions of North America. Although originally a disease of equines, recent VEE virus infections have occurred in epidemic proportions in human populations, suggesting a possible change in host range of the virus. These findings indicate this virus disease may become a problem as severe as the more well-known arbovirus infections of North America, such as those caused by eastern equine encephalitis (EEE), western equine encephalitis (WEE), and St. Louis encephalitis (SLE) viruses.

It is the purpose of this review to discuss the epidemiological and epizootiological aspects of this agent, particularly the distribution of the virus in man and lower animals and its possible

arthropod vectors. The implications of each of these hosts and vectors in the spread of the disease are considered. Related aspects of the virus, including etiology, means of detection and diagnosis, and possible control measures, which seemed pertinent to the review, are also described.

ETIOLOGY

Venezuelan equine encephalitis virus is one of nearly 20 ribonucleic acid (RNA) agents classified as Group A arboviruses, a classification based primarily on serological properties (14). The viruses of this group, although probably having common group antigens, are essentially distinct from one another. Two other viruses have been classified with the causative agent of VEE into the "Venezuelan equine encephalomyelitis complex" because of unusually high cross-reactivity with specific antisera in serum-neutralization, complement-fixation, and hemagglutination-inhibition tests (92). The other agents of this "complex" are the Mucambo and the Pixuna viruses. Since these three viruses are difficult to differentiate, they will be discussed together in this review.

Like other arboviruses of Group A, VEE is relatively small, showing a diameter of 40 to 45 m μ when ultrathin tissue sections were observed by electron microscopy (76, 77). The virus particles were described as being somewhat larger (65 to 80 m μ in diameter) in protamine sulfate-

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precipitated suspensions examined with an electron microscope (76, 77). In the electron micrographs of virus prepared by the latter method, the agent appeared to have a distinct core and a membrane. Other investigators have described an external envelope with a diameter of 60 to 70 $m\mu$ and a 30- to 40- $m\mu$ internal spherical nucleoid (58). The virus can be filtered through Berkefeld V and N filters and Seitz filters (78). The agent is not readily inactivated by Formalin and can be preserved by lyophilization or in 50% buffered glycerol at -70°C (102).

DETECTION AND DIAGNOSIS

Clinical Features

Until recently, VEE generally has been considered a mild disease in the human being. A recent outbreak in Venezuela, however, reportedly has taken the lives of at least 190 persons of nearly 32,000 with the disease (91). The exact incubation period is not known but is considered to be short, ranging from 2 to 5 days; the onset is usually very sudden. Symptoms may include headache, fever lasting from 1 to 4 days, malaise, chills, nausea or vomiting, and myalgia. Severe encephalitis or generalized systemic illnesses may occur. In rare instances, tremors, diplopia, and lethargy are also noted. Symptoms persist in mild cases for 3 to 5 days and for as long as 8 days in more serious attacks. After the general symptoms of the disease disappear, a prompt and apparently complete recovery usually takes place (13, 59, 66, 85, 96, 102). In the recent Venezuelan outbreak, Castillo (14a) and Rovira (84a) reported that fatalities occurred only in children of both sexes under 15 years of age, and that the clinical picture was often confused with the disease caused by the influenza virus.

Virus Isolation Procedures

The VEE virus can be readily recovered from blood and nasopharyngeal washings if taken during the acute phases of the illness in man or lower animals. The agent has been rather unique among the more well-known arboviruses in that it can be recovered for comparatively long periods of time from other areas of the body (e.g., bone marrow, spleen, liver, lung, kidneys, thymus, adrenals, brain, heart, lymph nodes) in addition to blood (104).

Isolations may be made by using a variety of laboratory animals including mice, rats, guinea pigs, hamsters, and monkeys. The animal of choice has been the mouse, which appears to be the most susceptible, particularly if inoculated intracerebrally. In these animals, the time of death has been found to be dose-dependent, and in-

fections are usually fatal (43). Intraperitoneal inoculations of mice also produce death, but Hearn (48) demonstrated an LD_{50} of $10^{1.5}$ less by the intraperitoneal than by the intracerebral route with the same virus. The average survival time in infant mice inoculated with the virus by either route has been shown to be markedly shorter than in similarly infected adult mice, although the mean survival time has been rather erratic in the latter animals when inoculated intraperitoneally (16). In recent years, suckling mice have been the animals most generally used for VEE virus isolation attempts (21, 31, 89, 91). Clinical signs of the disease in mice usually include hyperexcitability, hunching, and rough haircoat. The animals are usually severely depressed or paralyzed immediately prior to death.

Guinea pigs are quite susceptible to VEE virus infection when inoculated intraperitoneally, intracerebrally, or subcutaneously (3, 43, 62). When infected with the virus, the animals usually die within 2 to 4 days, with signs of paralysis evident just before death.

Recently weaned hamsters reportedly are nearly as susceptible as suckling mice to subcutaneous inoculation of VEE virus, and 5- to 10-week-old hamsters were used effectively in Mexico as "sentinels" for isolating the agent from mosquitoes (89).

Embryonated hens' eggs inoculated with the VEE agent via the yolk sac or chorioallantoic membrane routes readily become infected and die in approximately 15 to 48 hr, with large quantities of virus present (3, 60, 65).

Mussgay (75) described a technique for rapid isolation and identification of arboviruses by use of a plaque test in chick embryo cell cultures in which specific immune serum had been incorporated. With this technique, isolation and identification can be carried out in a single step. Sellers et al. (91) demonstrated the practicality of this test when a modification of it was used in studying an outbreak of VEE. A continuous line of baby hamster cells, BHK21 (68), which is susceptible to a number of arboviruses (72, 90), was used in these studies. Antiserum was either incorporated into the cell culture or mixed with the material suspected of containing the virus prior to addition to the cells. In the VEE outbreak studies, material tested included blood, serum, throat washings, brain suspensions, and crushed mosquitoes. Cytopathic effects (CPE) usually were apparent in 24 to 42 hr. Another possible modification of the above procedure is to test the cell culture fluid for hemagglutinins which appear 5 to 6 hr after infection (37). Immunofluorescence may also be used to detect VEE virus in cell cultures; in a

study using this method, virus was demonstrated in guinea pig heart cells 10 hr after infection (71).

Since viremia in VEE virus-infected animals has been found to be of rather short duration (rarely longer than 5 days), Smith et al. (97) suggested the use of bone marrow cells removed from the animal suspected of being infected. These cells, when cultured in vitro either alone or in combination with L cells, served both as a source of virus and as susceptible tissue supporting virus multiplication. Marked CPE was produced in virus-infected L cells in 48 to 72 hr. In studies in which this system was used with experimentally infected monkeys, isolation of VEE virus was carried out relatively late in the course of the infection, after the viremia had subsided. A number of advantages were cited for the use of marrow culture for VEE virus isolation: the system allows for isolation of a virus later in the course of infection, it provides a relatively simple cell culture system, and it may have application under field conditions when it is not possible to freeze specimens immediately. An additional advantage to all cell culture systems may be the inherent safety to laboratory personnel as compared with the use of laboratory animals.

Serological Techniques

Three serological tests are used routinely for arbovirus investigations. These tests, listed in order of specificity, include (i) the virus serum neutralization (SN) test, (ii) the complement-fixation (CF) test, and (iii) the hemagglutination inhibition (HI) test. General procedures for these tests are given by Hammon and Work (47) and Work (112). These procedures are applicable for essentially all arboviruses and have worked well with VEE.

Probably the earliest definitive study employing the SN test for VEE was that described by Kubes and Diamante (63), in which VEE, EEE, WEE, and "Argentine equine encephalitis" viruses were compared. No protection could be induced in mice against the VEE virus isolated by use of any of the other viruses, and the VEE agent would not protect against the other viruses. It was generally concluded from this early study that there was no relationship between these agents. Since this early work, the SN test has been used to some degree in the majority of the recent surveys for evidence of the VEE virus. Both mice and various susceptible cell cultures have been utilized as indicators of SN antibody titer in these studies. Arbovirus neutralizing antibodies often appear within a few hours after infection and may persist at significant titers throughout the life of the animal (101). One problem which has been encountered

in the use of the SN test is the loss of labile accessory factor which occurs on storage of sera, although this apparently occurs predominantly for Group B antibodies and can be eliminated by the addition of fresh normal serum or by using the intraperitoneal rather than the intracerebral route of inoculation if animals are used in the test (101).

At higher antibody titers, the CF test apparently has been quite specific for diagnosis of VEE. Casals (9), using CF tests, showed that this virus was unrelated to WEE, EEE, and SLE. Since "false-positives" are one of the most serious problems to be encountered when using the CF test, Casals (9, 10) recommended that human sera should be tested against several antigens simultaneously, and, if apparently nonspecific reactions are demonstrated, heating the sera at 65 C for 20 min should be tried. Complement-fixing antibodies generally persist in high titer for relatively short times, thereby providing a clue to the time of infection, especially when used in conjunction with HI procedures (101). A typical example of results when the CF test is used for detection of VEE antibodies can be seen in our survey for endemic diseases of the wildlife of west central Utah (105). In these studies, over 9,000 serum samples were tested simultaneously with VEE, EEE, WEE, and SLE antigens; CF antibody titers of 1:16 or greater for VEE, with no cross-reactions evident, were found in 57 serum specimens. Several of these sera also contained HI and specific SN antibodies to VEE. In addition to these apparently specific antisera, however, 17 other samples contained CF antibody which cross-reacted with one or more of the other antigens, and an additional 140 specimens were cross-reactive with all arbovirus antigens used.

By use of the HI test, a relatively high degree of cross-reactivity between VEE and other viruses has been observed. Casals and Brown (12) prepared an HI antigen from acetone and ether-extracted brain tissue of infected mice, and demonstrated hemagglutinins for chick erythrocytes associated with 13 viruses, including VEE. On the basis of the temperature and pH required for reaction, these viruses were separated into two groups, A and B. The VEE virus was included in Group A with EEE, WEE, and Sindbis viruses. Cross-reaction titers occurring among antisera specific to these other Group A viruses and VEE antigen were always at lower levels (three- to fourfold dilutions) than VEE immune serum titers. Casals (11) later separated the arboviruses into three groups on the basis of HI cross-reactivity. Always, VEE immune sera reacted to higher titer with homologous antigens than anti-

sera specific to the other agents in Group A. The Rockefeller Laboratory's techniques for the HI test were extensively described by Clarke and Casals (22). These investigators noted that lipid and lipoprotein are often nonspecific hemagglutination inhibitors in sera, and they described a method for the removal of this material by use of kaolin adsorption or acetone extraction. Holden et al. (50, 51) demonstrated the induction by acetone of an inhibitor which can be removed with protamine sulfate. Since acetone extraction is the method of choice for avian sera, protamine sulfate treatment of these acetone-extracted sera is therefore almost mandatory (50, 51). Phospholipids can also cause nonspecific inhibition in hemagglutination tests for VEE (80).

A number of surveys have been carried out in which the HI test has been used for VEE antibody determinations. The results reported by Sammartin and Duenas (87) are typical. This survey, which was carried out in Colombia, employed both the SN and HI tests. A greater number of positive sera were detected with the HI test than with the SN test (43 compared with 30); the HI titers were relatively high (greater than 1:80) in a number of cases. It is quite possible that a number of the sera containing demonstrable HI antibodies resulted from a cross-reaction induced by the presence of other viruses. In a discussion of serological surveys conducted in southern Florida, the confusingly broad serological HI overlap for Group A antigens was considered responsible for the reported high incidence of HI antibodies to VEE virus, previously unknown in this country (101). Later survey results, however, which included VEE virus isolations and detection of VEE HI and SN antibodies in the same area (21, 111), would seem to have reversed these earlier conclusions.

The HI test measures antibodies which appear somewhat later than the first detectable neutralizing antibodies, but which are usually produced prior to detectable CF antibodies (47). The detection of HI antibodies against other Group A arboviruses in sera obtained for serological surveys suggests that they may last a long time with little loss in titer, and are probably detectable throughout the life of the vertebrate host. Since HI antibodies are the most broadly reactive of the three antibody types being considered, however, a definitive interpretation of serological survey results by this test alone is difficult.

EPIDEMIOLOGY

Venezuelan equine encephalitis has been primarily a disease of equines and other lower animals, although occasionally the agent has

infected man. The disease was apparently first noted in equines of Colombia in 1935 when reported from the Department of Valle, Tolima, Bolivar, and Huila (1). Early in 1936, it was found in equines of some portions of the Department of Magdalena and later in the Colombia portions of the La Guajira Peninsula (73). The disease was first reported in Venezuela in the La Guajira Peninsula (61) in 1936, and it spread from this area to practically the entire country in the course of 1937 to 1939 (3, 65). In subsequent years, the disease assumed more of a sporadic character both in Colombia and Venezuela, appearing in epizootic form in horses and mules in 1942 and later in man, in 1952 and again in 1962, as an alarming outbreak of encephalitis (5a, 84a, 86a, 89, 91).

From the 1942 Venezuelan epizootic, the virus apparently spread to Trinidad, having been isolated from sick equines on this island in 1943 and 1944 (62) and possibly causing fatalities in two humans during the epizootic (41, 82, 106, 107).

Evidences of the infection have subsequently been found in Ecuador (2, 24, 67, 100), British Guiana (107), Panama (37a, 78a, 89, 107), Argentina (5, 110), French Guiana (36), Surinam (53, 55, 108), Mexico (89, 73a), Brazil (6, 15, 16, 17), Curacao, West Indies (32), and portions of the United States: Louisiana (56), Florida (21, 111), and Utah (105). A survey of sera from residents of Antigua, Jamaica, Barbados, St. Lucia, and St. Vincent Islands of the West Indies revealed no VEE neutralizing antibody (28, 29, 30, 33, 34). Sera from south Florida patients with undiagnosed infectious disease were recently tested for a spectrum of arbovirus HI antibodies. No VEE antibody could be detected (79). Virus isolates from Brazil were later shown to be different from the original VEE virus isolations; these viruses were named Mucambo and Pixuna and are considered part of the "VEE complex" (92).

A summary to date of the approximate geographical distribution of VEE is shown in Fig. 1.

Natural Distribution in Man

Since VEE virus infections in humans are of the greatest concern from a public health standpoint, a breakdown of the incidence in human populations is of interest. Until 1942, VEE appeared to be detected only in equines, the disease produced in horses resembling that caused by EEE and WEE viruses. In 1943, however, two human fatalities in Trinidad were attributed to VEE (41, 82). Later analyses of these reports have indicated the earlier conclusions to be equivocal (107), although specific neutralizing

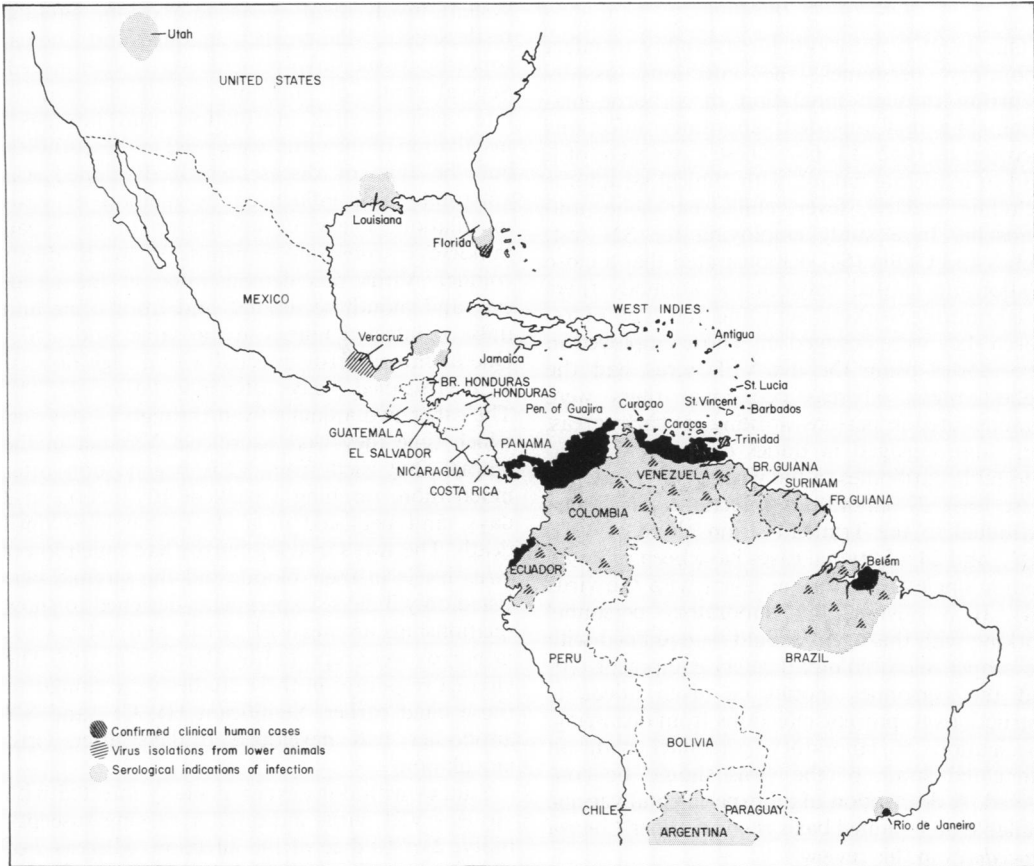


FIG. 1. Summary of the approximate geographical distribution of Venezuelan equine encephalitis virus.

antibodies were demonstrated in the blood of persons in the area (106). In 1944, the disease in man was of such a nature that Gallia and Kubers (38a) stated that it "never occurred in such a form as to be able to draw the attention of public health authorities." A dengue-like fever of at least 70 persons in Colombia was shown in 1952 to be caused by the VEE virus, as determined by isolation and identification of the agent from blood samples (88). After an apparent subsidence, the disease again appeared in Colombia in 1955 and 1957 as an acute febrile illness. The agent was once again recovered from the blood of patients, and a number of human sera taken from the area contained significant HI antibody titers against the virus (45). Subsequently, in 1961 and 1962, a severe outbreak of the disease recurred in Colombia and also in northwest Venezuela and in Panama. In Panama, several hundred people became infected, whereas in Venezuela nearly 32,000 cases of VEE were reported, with at least 190 fatalities (91). The

fatality rate of 6,000 human cases in Venezuela has been reported at 0.6% (103). Early reports of the outbreak indicated all of the fatal cases to be in children under 15 years of age (5a, 84a).

Inapparent VEE virus infections in man, as determined by specific neutralizing antibodies, have occurred in Argentina (5), Curacao, N.W.I. (32), Trinidad (106), Surinam (53, 55, 108), the coastal region of Ecuador (2, 24), northeastern Venezuela (54, 86a), Mexico (73a), and in Seminole Indians of Florida (111). Floch et al. (36) reported the occurrence of VEE antibodies in sera from humans of French Guiana, but the same sera were also reactive to SLE virus. Sera of residents of Rio de Janeiro, Brazil (6), and San Vincente de Chucuri, Colombia (45), reportedly contained HI antibodies. Specific neutralizing antibodies, as well as virus isolates, were obtained from residents of Belém, Brazil (16, 17), the viruses isolated later being shown to be other members of the "VEE complex."

Laboratory Infections

This agent is notorious for its ability to infect laboratory personnel, the infections usually occurring through inhalation of airborne material. The first reported laboratory infections occurred in 1942 (13, 66). These cases were of a mild to moderate nature, with clinical symptoms including malaise, fever, chills, backache, and headache. In a study employing the SN test, Kubes and Gallia (64) demonstrated that a close relationship existed between the antibody titers in laboratory workers' sera and the type of research they were engaged in. Those employed in laboratories using the live VEE virus had the highest titers, whereas occasional titers were demonstrated in persons working in nearby areas, and those engaged in duties elsewhere had no detectable antibodies to the virus.

At least 27 laboratory infections have been attributed to the Trinidad strain alone of VEE (107), and 24 additional cases have been reported from a single laboratory accident with the virus (94). It is obvious that laboratory personnel working with this agent should be equipped with the proper microbiological safety equipment (70) and the personnel should be given adequate instructions to prevent infections from occurring. Vaccination of laboratory personnel with an attenuated strain of the virus should be considered. A description of an experimental vaccine available on a limited basis is given under *Control Procedures* in this review.

Distribution in Domestic Animals

The VEE virus has been isolated from naturally infected horses (62, 65, 82, 95, 99), mules (62, 82), and donkeys (40, 91). Neutralizing and HI antibodies of significant titer have been found in sera from dogs, goats, pigs, sheep, and cattle (91). Apparently specific complement-fixing antibodies have been found in the sera of 7 of 4,719 range cattle of Utah (Thorpe, unpublished data). These bovine VEE antibody titers were 1:16 to 1:256; no evidence was seen of cross-reactions with EEE, WEE, SLE, psittacosis, or rickettsial antigens.

The virulence of the agent is apparently variable in equines. Kissling et al. (57) reported infections ranging in severity from subclinical to fatal in experimentally infected horses. Virus titers in these infected horses ranged from 10^4 to $10^{7.5}$ mouse intraperitoneal LD_{50} (MIPLD₅₀) at 18 to 108 hr after inoculation, and the virus persisted in the blood until death, or from 17 through 144 hr in surviving animals. The clinical symptoms in these animals included depressiveness, high temperature, and occasionally central nervous

system involvement (chewing motion, circling, restlessness). Pancreatic lesions and hematopoietic tissue involvement were often noted. Several routes of infection, including mosquito bite, subcutaneous inoculation, and intranasal installation of the virus, were used in these studies, but the type of disease which developed was apparently not influenced by method of virus inoculation. The virus was shown to be spread in horses by mosquito transmission and by direct contact. Virus was demonstrated in the nasal, eye, and mouth secretions, and from urine and milk of infected horses in the same study (57). One of three burros infected with a high dose of VEE virus died 5 days after inoculation (43, 44). The other two animals lived through 14 days, at which time they were sacrificed. Viremia in the animal that died from VEE began soon after inoculation, reaching a peak of 10^7 MIPLD₅₀ in 2 days, and persisted at a detectable level until death. The two surviving burros had much lower virus levels in their blood, and the viremia persisted only 3 days. Experiments designed to show relationships among VEE, EEE, and WEE virus in burros were reported by Byrne et al. (8). The viremia responses in these studies were similar to those cited earlier. Significant HI, CF, and SN antibodies had developed 6 days after virus inoculation.

Studies with dogs experimentally infected with virulent VEE virus indicated a varying susceptibility; 2 of 10 died of the infection, whereas 7 of 10 showed no frank clinical signs of illness. Fever, leucopenia, and a low-titer viremia of short duration were observed (103). In a later study, contact transmission of the virus from infected to noninfected dogs was demonstrated (26). In this latter study, 6 of 10 challenged animals died of the virus infection. Short-term viremia with titers as high as $10^{8.5}$ MIPLD₅₀ was demonstrated from the challenged dogs, and *Aedes triseriatus* mosquitoes were found capable of receiving the virus after feeding on these animals.

Distribution in Wild Mammals

Evidence is mounting to indicate that wild mammalian hosts may be important in the maintenance of the VEE agent in nature. The virus has been isolated from a number of wild mammals to date, including tufted capuchin monkeys, *Cabus apella* (16), the spiny rat, *Proechimys* sp. (16), the forest spiny pocket mouse, *Heteromys anomalus* (31), the terrestrial rice rat, *Oryzomys laticeps velutinus* (31), and short-tailed cane mouse, *Zygodontomys brevicauda* (31). In addition, antibodies (SN or HI) have been found in the opossum, *Didelphis* sp. (91),

the cotton mouse, *Peromyscus gossypinus* (21), and the cotton rat, *Sigmodon hispidus* (21). In a survey for CF antibody to the VEE virus and other agents in various wild mammals native to the western desert area of the United States, the sera of eight species (58 specimens) were considered positive at low (1:16 to 1:64) titers (105). Cross-reactions with other Group A arboviruses tested simultaneously were not evident in these sera. The seropositive animals included the deer mouse (*Peromyscus maniculatus*), the western harvest mouse (*Reithrodontomys megalotis*), the chisel-toothed kangaroo rat (*Dipodomys microps*), the white-tailed antelope squirrel (*Citellus leucurus*), the black-tailed jackrabbit (*Lepus californicus*), the nuttall and desert cottontail (*Sylvilagus nuttallii* and *S. audubonii*), and the kit fox (*Vulpes macrotis*). Thirty-eight of these positive serum samples, involving all species except the harvest mouse, also had VEE HI antibody titers of 1:40 or greater. Nine of the 58 samples were tested for neutralizing antibodies; 4 of these sera (from one deer mouse and three cottontails) were considered to have significant SN indices. In 157 other serum samples, cross-reactions with one or more other arbovirus antigens were demonstrated by use of the CF test. These cross-reactive samples were not tested with the HI or SN techniques.

The tufted capuchin monkey is apparently a host for the viruses of the "VEE complex." Causey et al. (16) hung cages containing the animals in the Amazon jungles. They then attempted to isolate virus from blood samples taken daily. In this way, a large number of isolates of VEE virus (later shown to be Mucambo virus) were made. The viremia in these monkeys was found to occur within 4 days after infection, persisting no longer than 2 weeks. The virus titers during this time were well above the threshold value required for mosquitoes feeding upon the monkeys to obtain an infectious blood meal.

Gleiser et al. (42, 43) experimentally infected rhesus monkeys with the VEE virus and noted the pathological signs in the animals included reversible injury to the lymphatic and nervous system and inflammatory lesions in the central nervous system. Viremia occurred within 1 day after inoculation, reaching a peak in 24 hr and persisting at a high level for 3 days, then declining to a negligible level by 6 days. A minimal clinical response characterized by a diphasic fever was apparent in these animals.

Although little is known of the disease in wild rodents except for what has been stated above, laboratory animals such as mice, guinea pigs, hamsters, and rats are extremely susceptible to

VEE virus infection, dying readily of the disease and producing high-titer, persistent viremia. The studies with these animals were described under *Virus Isolation Procedures*.

Bats appear experimentally to be excellent hosts for the VEE agent. Corristan et al. (25) reported that four species (big brown bat, *Eptesicus fuscus*, little brown bat, *Myotis lucifugus*, eastern pipistrel, *Pipistrellus subflavus*, and long-eared bat, *Corynorhinus rafinesquii*) were readily infected experimentally when exposed to the virus by either the intranasal or intraperitoneal routes. The infectious dose for each bat species was approximately equivalent to that of Swiss-Webster mice infected by the same routes. When inoculated with 25 MIPLD₅₀ of VEE virus, blood virus titers in *E. fuscus* and *M. lucifugus* attained peaks of at least 10⁶ MIPLD₅₀ within 48 hr, with significant titers persisting for at least 26 days. At intervals during this time, these titers were well above the threshold values usually required to infect mosquitoes. Low titers of the virus persisted in these bats for at least 90 days when at a hibernating temperature (10 C), and when the temperature was raised, the virus titers rose rapidly. The infection was apparently not lethal for these mammals.

A summary of the animals which have been reported to be susceptible to the VEE virus is shown in Table 1.

Distribution in Birds

The VEE virus is different from EEE and WEE viruses, other agents of the Group A arboviruses, in that it seems to multiply better in mammals than in birds. Until recently, there have been few complete reports of isolations of the agent from wild-caught birds. Samper and Soriano-Lleras (86) stated that the pigeon was the only known animal to be naturally infected at the time of their studies. These investigators showed experimentally that VEE virus could not be demonstrated after three passages through pigeons. Briceno Rossi (5a) isolated the agent from a chicken and a rooster taken from an endemic area of VEE in Venezuela during the recent epidemic of the disease in that country. A number of wild fowl caught in the same area contained no signs of VEE virus infection. The same investigator demonstrated chicks less than 1 month old to be fatally susceptible to experimental infection with the virus; experimentally infected chickens older than 1 month produced antibodies to the virus but were not clinically ill and no viremia was detected. Kissling et al. (56) reported the presence of neutralizing antibodies

to VEE virus in the sera of a number of birds caught in the swamplands of Louisiana. Among these serologically positive birds were the white ibis, *Guara alba*, and the yellow-crowned night heron, *Nyctanassa violacea*, which migrate from South and Central America during the summer. All other birds having neutralizing antibodies to the virus were considered permanent residents of the area. In this study, titers were usually low to moderate (neutralization indices of 22 to 79 or greater), in comparison to relatively high titers to EEE and WEE viruses (neutralization indices of 162 to 630) in the same species of birds. Of the permanent resident birds having antibody to the VEE agent, a high percentage had EEE or WEE titers also. Recently, Galindo et al. (37a) reported isolations of the virus from 10 species of birds caught in northwestern Panama (Table 1). Several of these birds are known to migrate to North America.

Chamberlain et al. (19) infected wild birds with VEE virus by subcutaneous inoculation and by mosquito bite. All species of birds tested were relatively resistant, the English and white-throated sparrows (*Passer domesticus domesticus* and *Zonotrichia albicollis*) being more susceptible than pigeons (*Columba livia*), cardinals (*Richmondea cardinalis*), and mourning doves (*Zenaidura macroura*). English sparrow-mosquito-English sparrow transmission could be demonstrated by use of the *Aedes triseriatus* mosquito. Virus levels were usually quite low in all of the tested birds, the English sparrow having viremia as early as 17 hr, with peak titers of $10^{3.8}$ to $10^{5.0}$ MIPLD₅₀ at 40 to 64 hr, and persisting up to 5 to 7 days (inoculated with 3.2 MIPLD₅₀ doses of virus). White-throated sparrows, mourning doves, cardinals, and pigeons were inoculated with 346 MIPLD₅₀ of virus; viremia was first observed after 18 hr and persisted up to 162 hr after inoculation. Virus titers varied, but were of significant levels (at least $10^{2.6}$ MIPLD₅₀) in pigeons, sparrows, and cardinals; essentially no virus, however, could be recovered from the blood of experimentally infected doves. When the same birds were inoculated with a higher dose (2,000 MIPLD₅₀), viremia was shorter in duration, reaching a maximum in approximately 96 hr, with no higher peak titers than were previously observed. A pigeon infected through the bite of a mosquito had a titer of $10^{1.6}$ MIPLD₅₀ at 144 hr after the initial infection. The peak titer in this bird was not observed until 120 hr. Infections in all birds were symptomless. Miller (72a) had reported pigeons to be susceptible to respiratory infections with the VEE virus. Viremia titers approached 10^5 MICLD₅₀ units per ml of blood. The implication of this

latter study is the possibility of virus transmission from bird to bird by the respiratory route, perhaps from aerosolization of excreted virus.

A recent survey of 71 sera of wild birds from Venezuela by Sellers et al. (91) failed to yield any positive for VEE antibody by either the SN or HI test, although significant titers were demonstrated in sera of humans, livestock, and wild mammals from the same geographical area. Five buzzards caught by Gilyard (41) during the epizootic in Trinidad were apparently uninfected with the virus. The previously cited CF antibody study in wildlife of Utah included tests on 727 bird sera of 62 different species (93, 109), but only a single serum sample taken from a sage thrasher (*Oreoscoptes montanus*) was considered to have a significant CF antibody titer to VEE virus (105). This specimen was also found to have HI antibodies at a titer of 1:160. Most of the birds from the area are migrants, with only 20 species of approximately 270 known to be permanent residents. The sage thrasher arrives in the spring, nests and departs in the fall.

Possible Arthropod Vectors

A significant number of mosquito species have been shown to be capable of VEE virus transmission and have yielded isolations of the virus in nature. These mosquito species are summarized in Table 2. No other arthropods have as yet been implicated with the disease.

In Trinidad during the 1943 epizootic, Gilyard (40, 41) found *Mansonia titillans* to comprise 90% of the mosquitoes caught in stable traps set in the area. The VEE agent was isolated from a number of these mosquitoes at the time, and this species was thus implicated by Gilyard as the primary vector in the epizootic. This mosquito was also found in endemic areas of Ecuador (67), and the species reportedly will transmit the virus experimentally (35, 57). No apparent transovarial passage of VEE virus occurred from infected *M. indubitans* and *M. titillans* (57), but virus titers of greater than 10^8 were demonstrated in eggs laid by infected *M. perturbans* (20).

The *Aedes* sp. also have been implicated as vectors of VEE virus. *Aedes taeniorhynchus* was caught in small numbers during the Trinidad studies (40), and in Ecuador (67). It is capable of long flights, has wide distribution, and has been suspected of carrying the VEE virus from epidemic areas in Venezuela to Trinidad (40). During the recent VEE outbreak of humans in Venezuela, *A. taeniorhynchus* appeared to be the most frequent carrier of the organism (91). Eklund (35) has shown that the species is capable

TABLE 1. *Animals known to be susceptible to infection with Venezuelan equine encephalitis virus*

Species	Common name	Type of study ^a	Reference ^b
MAMMALIA			
<i>Bovis</i> sp.	Cow	N, H, C	Sellers et al. (91), Thorpe (<i>unpublished data</i>)
<i>Cabus apella</i>	Tufted capuchin monkey	I, E	Causey et al. (16), Causey and Theiler (17)
<i>Canis familiaris</i>	Dog	N, H	Sellers et al. (91)
<i>C. familiaris</i>	Dog	E	Taber et al. (103)
<i>Capra</i> sp.	Goat	N, H	Sellers et al. (91)
<i>Cavia cobaya</i>	Guinea pig	E	Beck and Wykoff (3)
<i>Citellus leucurus</i>	Antelope ground squirrel	C	Thorpe et al. (105)
<i>Corynorhinus rafinesquii</i>	Lump-nosed bat	E	Corristan et al. (25)
<i>Didelphis</i> sp.	Opossum	N, H	Sellers et al. (91)
<i>Dipodomys microps</i>	Chisel-toothed kangaroo rat	C, H	Thorpe et al. (105, <i>unpublished data</i>)
<i>Eptesicus fuscus</i>	Big brown bat	E	Corristan et al. (25)
<i>Equus asinus</i>	Mexican burro	E	Gleiser et al. (43)
<i>E. caballus</i>	Horse	I	Kubes and Rios (65)
<i>E. caballus</i>	Horse	E	Kissling et al. (57)
<i>Equus</i> sp.	Donkey	I	Baquérizo and Marmol (2)
<i>Equus</i> sp.	Donkey	E	Gilyard (40)
<i>Equus</i> sp.	Mule	I	Kubes (62), Randall and Mills (82)
<i>Heteromys anomalus</i>	Forest spiny pocket mouse	I	Downs et al. (31)
<i>Lepus californicus</i>	Black-tailed jackrabbit	C, N, H	Thorpe et al. (105, <i>unpublished data</i>)
<i>Macaca mulatta</i>	Rhesus monkey	E	Hearn (48)
<i>Mus musculus</i>	Laboratory mouse	E	Gleiser et al. (43), Hearn (48)
<i>Myotis lucifugus</i>	Little brown bat	E	Corristan et al. (25)
<i>Oryctolagus cuniculus</i>	Laboratory rabbit	E	Hearn (48)
<i>Oryzomys laticeps</i>	Terrestrial rice rat	I	Downs et al. (31)
<i>Ovis</i> sp.	Sheep	N, H	Sellers et al. (91)
<i>Peromyscus gossypinus</i>	Cotton mouse	N, H	Chamberlain et al. (21)
<i>P. maniculatus</i>	Deer mouse	C, N	Thorpe et al. (105)
<i>Pipistrellus subflavus</i>	Long-eared bat	E	Corristan et al. (25)
<i>Proechimys</i> sp.	Spiny rat	I	Causey et al. (16)
<i>Rattus norvegicus</i>	Laboratory rat	E	Causey et al. (16)
<i>Reithrodontomys megalotis</i>	Western harvest mouse	C	Thorpe et al. (105)
<i>Sigmodon hispidus</i>	Cotton rat	N, H	Chamberlain et al. (21)
<i>Sus</i> sp.	Pig	N, H	Sellers et al. (91)
<i>Sylvilagus audubonii</i>	Audubon cottontail rabbit	C, H	Thorpe et al. (105, <i>unpublished data</i>)
<i>S. nuttallii</i>	Nuttal cottontail rabbit	C	Thorpe et al. (105, <i>unpublished data</i>)
<i>Vulpes macrotis</i>	Kit fox	C, H	Thorpe et al. (105, <i>unpublished data</i>)
<i>Zygodontomys brevicauda</i>	Short-tailed cane mouse	I	Downs et al. (31)
AVES			
<i>Butorides virescens</i>	Green heron	I	Galindo et al. (37a)
<i>Columba livia</i>	Domestic pigeon	E	Chamberlain et al. (19), Samper and Soriano-Lleras (86)
<i>Corvus brachyrhynchos</i>	Crow	N	Kissling et al. (56)
<i>Crotophaga sulcirostris</i>	Groove-billed ani	I	Galindo et al. (37a)
<i>Cyanocitta cristata</i>	Blue jay	N	Kissling et al. (56)
<i>Dendroica coronata coronata</i>	Myrtle warbler	N	Kissling et al. (56)
<i>Florida caerulea</i>	Little blue heron	I	Galindo et al. (37a)
<i>Guara alba</i>	White ibis	N	Kissling et al. (56)

TABLE 1.—Continued

Species	Common name	Type of study ^a	Reference ^c
<i>Icterus prothemelas</i>	Black-cowled oriole	I	Galindo et al. (37a)
<i>Megaceryle alcyon alcyon</i>	Eastern belted kingfisher	N	Kissling et al. (56)
<i>Melospiza melodia</i>	Song sparrow	E	Chamberlain et al. (19)
<i>Myiozetetes granadensis</i>	Gray-capped flycatcher	I	Galindo et al. (37a)
<i>M. similis</i>	Social flycatcher	I	Galindo et al. (37a)
<i>Nyctanassa violacea</i>	Yellow-crowned night heron	N	Kissling et al. (56)
<i>Oreoscoptes montanus</i>	Sage thrasher	C, H	Thorpe et al. (105, unpublished data)
<i>Passer domesticus domesticus</i>	English sparrow	E	Chamberlain et al. (19)
<i>Quiscalas quiscula</i>	Purple grackle	N	Kissling et al. (56)
<i>Ramphastos sulfuratus</i>	Keel-billed toucan	I	Galindo et al. (37a)
<i>Ramphocelus passerinii</i>	Scarlet-rumped tanager	I	Galindo et al. (37a)
<i>Richmondia cardinalis</i>	Cardinal	N	Kissling et al. (56)
<i>R. cardinalis</i>	Cardinal	E	Chamberlain et al. (19)
<i>Turdus grayi</i>	Clay-colored robin	I	Galindo et al. (37a)
<i>Zenaidura macroura</i>	Mourning dove	E	Chamberlain et al. (19)
<i>Zonotrichia albicollis</i>	White-throated sparrow	E	Chamberlain et al. (19)

^a C = Complement-fixing antibody to the VEE virus demonstrated in serum from captured animal. E = Susceptibility based on studies in the laboratory. H = Hemagglutination inhibition antibody to the VEE virus demonstrated in serum from captured animal. I = Isolation of VEE virus from captured animal. N = Neutralization antibody to the VEE virus demonstrated in serum from captured animal.

^b Earliest or most complete study used as reference.

of transmitting the VEE agent in the laboratory. *A. serratus* was found in great numbers in Ecuador (67), Brazil (16), and Trinidad (31), and in lesser numbers in the endemic areas of Venezuela (91); VEE virus isolations were made from these mosquitoes in the latter three countries. Virus was isolated from *A. scapularis* caught during the Venezuelan epidemic (91), and the mosquito also was trapped in Brazil, although no virus isolates were made in the latter case (16). Experimentally, *A. aegypti*, *A. albopictus*, *A. geniculatus*, and *A. triseriatus* mosquitoes are reportedly capable of transmission of the virus (20, 26, 57, 84).

Mosquitoes of the genus *Culex* (subgenus *Melanoconion*) have yielded the only VEE virus isolations made in the United States (21). A survey in Colombia (46), Trinidad (31), and in Panama (37a, 78a) yielded isolates from *Culex* sp. mosquitoes, but Sellers et al. (91) were unable to isolate the agent from small numbers of this species caught in Venezuela. Eklund (35) reported that the *Culex* sp. transmitted the disease agent experimentally.

Other genera of mosquitoes of possible importance as vectors for VEE virus transmission include *Anopheles*, *Haemogogus*, *Psorophora*, and *Sabethes*. *Anopheles neomaculipalpus* was collected in moderate numbers from stable traps during the Trinidad outbreak, and one mosquito of this species reportedly transmitted the VEE virus from one donkey to another (40). One pool

of *A. aquasalis* of 40 tested yielded an isolate of VEE virus in Venezuela (91). Pools of *Anaphelini* sp. yielded isolates of the virus in Panama (37a, 78a). Pixuna virus (one of the "VEE complex") reportedly was isolated from *A. nimbus* in Belem, Brazil (18). Causey et al. (16) recovered one of the "VEE complex" viruses from *Haemogogus* sp. mosquitoes in Brazil; small numbers of this species were caught in Venezuela during the recent VEE outbreak in the country, but no virus isolates were reported from them (91). *Psorophora ferox* yielded isolates of the VEE virus or viruses of the "VEE complex" in Brazil (16), Colombia (46), and Trinidad (31). The VEE virus was recovered from 1 of 14 pools of *P. confinnis*, but not from pools of *P. cilipes*, *P. ferox*, or *P. varipes* caught in Venezuela (91). Female *P. ferox* mosquitoes were shown experimentally to transmit VEE to healthy guinea pigs 14 days after infection (20). Virus isolates of the "VEE complex" were recovered from *Sabethes* sp. in Brazil by Causey et al. (16).

CONTROL PROCEDURES

Control of diseases such as VEE can theoretically be approached by a variety of techniques: (i) elimination of the arthropod vectors, (ii) the use of vaccines, (iii) decontamination of surroundings, and (iv) the use of chemotherapy.

The elimination of the arthropod vectors (only mosquitoes to date) would appear to be the most effective means for control on the basis of past

experiences with related viruses. A mosquito eradication program carried out in an endemic area of Venezuela at the height of the 1962 VEE outbreak was thought to be a significant factor in halting the advance of the disease in the area (14a).

A Formalin-inactivated vaccine prepared in chick embryos has been used in Venezuela for prevention of VEE in horses (65). A partially purified vaccine reportedly has produced high levels of antibody and has been used for the protection of laboratory personnel (81). A vaccine prepared later, consisting of VEE, EEE, and WEE, has also been used (69). These vaccines, however, apparently contained active virus which was undetected in laboratory animal safety tests, since a significant number of individuals inoculated with these vaccines developed symptoms of the disease, and in some cases yielded isolates of the virus (96, 102). Attempts to use such Formalin-prepared vaccines have consequently been discouraged. Attenuation of the virus has been achieved in certain cell cultures (4, 48, 74), and an active attenuated vaccine has subsequently been produced. Gochenour et al.

(44) induced a solid immunity in burros against a challenge dose of VEE, using an attenuated strain of the VEE virus. The virulence of the agent was not restored by passage in the burro. Similar results were observed with an attenuated virus strain in dogs (103). McKinney et al. (70) employed this attenuated virus for the immunization of laboratory personnel, and followed the course of viremia and the antibody response. After 1 to 2 years, antibody titers were still at high levels in immunized persons. An experimental vaccine comprised of this attenuated virus is available from the U.S. Army Medical Unit, Fort Detrick, Frederick, Md., for governmental agencies and research institutions. Administration of the vaccine is "limited to persons who are considered to be 'at risk' to infection because of occupation or other similar circumstances" (*Personal communication*, Dan Crozier, U.S. Army Medical Unit, Fort Detrick, Frederick, Md.).

Hyperimmune serum therapy during the first 10 to 14 hr is recommended in the case of known laboratory infections (47).

TABLE 2. *Known species of mosquitoes from which Venezuelan equine encephalitis virus has been isolated*

Species	Method ^a	Reference
<i>Aedes aegypti</i>	E	Roubaud et al. (84)
<i>A. albopictus</i>	E	Roubaud et al. (84)
<i>A. geniculatus</i>	E	Roubaud et al. (84)
<i>A. serratus</i>	I	Causey et al. (16), Downs et al. (31), Sellers et al. (91)
<i>A. scapularis</i>	I	Sellers et al. (91)
<i>A. taeniorhynchus</i>	I	Eklund (35), Sellers et al. (91)
<i>A. taeniorhynchus</i>	E	Eklund (35)
<i>A. triseriatus</i>	E	Chamberlain et al. (19), Kissling et al. (57)
<i>Anopheles aquasalis</i>	I	Sellers et al. (91)
<i>A. nimbus</i>	I	Chamberlain (18)
<i>A. neomaculipalpus</i>	I	Gilyard (40)
<i>Anophelini</i> sp.	I	Galindo et al. (37a), Peralta and Shelokov (78a)
<i>Culex quinquefasciatus</i>	I	Galindo et al. (37a)
<i>C. quinquefasciatus</i>	E	Eklund (35)
<i>C. taeniopus</i>	I	Galindo et al. (37a), Peralta and Shelokov (78a)
<i>C. vomerifer</i>	I	Galindo et al. (37a), Peralta and Shelokov (78a)
<i>Culex</i> sp.	I	Chamberlain et al. (21), Downs et al. (31), Groot et al. (46)
<i>Haemogogus</i> sp.	I	Causey et al. (16)
<i>Mansonia indubitans</i>	E	Kissling et al. (57)
<i>M. perturbans</i>	E	Chamberlain et al. (20)
<i>M. titillans</i>	I	Gilyard (40)
<i>M. titillans</i>	E	Kissling et al. (57)
<i>Psorophora confinnis</i>	I	Sellers et al. (91)
<i>P. ferox</i>	I	Causey et al. (16), Downs et al. (31), Groot et al. (46)
<i>P. ferox</i>	E	Chamberlain et al. (20)
<i>Sabethes</i> sp.	I	Causey et al. (16)

^a E = Mosquito shown capable of VEE virus transmission in the laboratory. I = Virus isolated from captured mosquitoes.

Since the VEE virus has been shown capable of being transmitted by direct contact, decontamination of the surroundings is of importance, particularly as a means of protecting laboratory personnel. Propylene glycol, glycolic acid, thioglycolic acid, thiourea, and methyl thioglycolate are effective for inactivation of the VEE virus (27, 38, 49), and a variety of other standard disinfectants have been shown to be highly effective against other equine encephalitides (7).

No drug has been reported to show significant activity *in vivo* against VEE virus; hence, specific chemotherapy is not possible at present.

SUMMARY AND OUTLOOK

An overview of what has been discussed concerning VEE reveals two observations of particular interest. The first is that the geographical range of the disease agent is possibly extending, the virus being first isolated in Colombia, then apparently spreading through the forested parts of coastal South America, and now appearing in North America in coastal areas where the agent might be expected to be detected (i.e., Florida, Eastern Mexico, Louisiana). It is possible that evidence of the virus has been discovered in these extended areas because the agent is now being looked for in these areas for the first time, but an examination of the epidemiological data would indicate that in certain areas an extension in geographic range has been obvious. Interestingly, an extension in range in the reverse direction has recently been observed for SLE (23). A second observation of considerable importance is a possible change in host range which has apparently occurred in the VEE virus. For 20 years, the agent was considered chiefly the cause of a disease of equines, with clinical diseases in the human rarely, if ever, occurring. Recently, however, the disease caused by an immunologically similar virus in Venezuela, Colombia, and Panama resulted in severe human infections, with at least 190 fatalities recorded. These two observations concerning VEE would imply that a definite public health menace could be imminent to both North and South America.

Like most of the mosquito-borne encephalitis viruses, the VEE agent apparently has a complex natural infection cycle, possibly involving several vertebrate hosts, as well as mosquitoes and perhaps other arthropod vectors. Unlike the related Group A arboviruses such as WEE and EEE viruses, however, the available evidence would indicate that birds play a lesser role in the infection cycle of the VEE virus and that mammals may be the primary natural hosts. This

assertion is based on reports that the viremias of experimentally infected birds have been lower than those usually found in mammals, and that transmission from one mammalian host to another by the mosquito has been more readily demonstrated. It is difficult, however, to extrapolate the findings of laboratory studies carried out under controlled conditions to the natural ecology of the disease, and birds should definitely be considered to play some role in the transmission cycle of the virus, particularly in view of the recent isolations of the virus made from migratory birds (37*a*). Often in both birds and mammals, the VEE virus infection is inapparent, which is a definite advantage for a reservoir host. Another factor to be considered is the rate of death of both mammalian and avian hosts during a severe epizootic of the disease agent. Chamberlain et al. (19) have pointed out that the avian replacement rate is faster than the replacement rate for the larger mammals, and thus "birds might be involved in an endemic maintenance of infection, furnishing foci for periodic epidemic spread." Man would appear to be an incidental host, although, during times of an epidemic, he could become a significant vertebrate element in the transmission cycle.

The specific VEE neutralizing antibodies demonstrated in the sera of birds in Louisiana which had migrated from South America would suggest that migrant birds may well have been the means by which the virus was introduced into the United States. The few evidences of the disease in Utah would appear illogical except that an extensive bird refuge, which hosts large numbers of migrant birds, is located in north central Utah, and over 250 species of nonpermanent birds have been observed in the area from which the seropositive specimens were taken.

The mosquito is obviously the vector for usual transmission of the VEE virus from one host to another, and, because of the wide variety of mosquito species from which the agent has been isolated, conceivably the disease could become a problem in many parts of the world. Several species of *Aedes* (*A. aegypti*, *A. scapularis*, *A. taeniorhynchus*, *A. triseriatus*), *Mansonia* (*M. indubitans*, *M. perturbans*, *M. titillans*), *Psorophora* (*P. confinnis*, *P. ferox*), and *Culex* mosquitoes which have been implicated as vectors of the VEE virus are known to be present in significant numbers in areas of North America (8*a*). The fact that this virus can also be transmitted by direct contact confuses the natural infection cycle of the agent, but lends weight to the eventuality of a possible world-wide distribution of the virus.

A combination of suitable climate, vegetation, and topography is apparently a critical factor in outbreaks of VEE; the virus essentially has been limited to the low lands of the rain-forested areas of the tropics to date. The recent alarming outbreak of the disease in Venezuela and surrounding countries came at a time when the total rainfall was approximately double the average for the preceding 5 years. Such flooding provided excellent breeding conditions for mosquitoes and caused a crowding of animals on the unflooded land areas (14a, 84a, 91). Although temperate conditions are dominant in the VEE endemic areas and over-wintering is not a problem, there is no reason to believe the virus would not be capable of persisting in various animal hosts through cold seasons. It is conceivable also that an overwintering host such as poikilothermic animals, which has been suggested for WEE virus (39), could maintain the virus for long periods of time. Transovarial passage of the virus through certain mosquitoes, which has been demonstrated experimentally (20), would indicate an additional means for virus survival during winter months.

Suggested further research would be centered around the manner of transmission of the VEE virus from South America to North America, further elucidation of the infection cycle in nature, and investigations of the antigenic differences among the strains of VEE virus. Extensive surveys of wild animal populations, including poikilothermic animals, for viremic status, and antibody studies in human and lower animal populations of areas between the United States and the northern portions of South America seem to be warranted. Such areas should primarily include Central America and the islands of the West Indies, as well as other areas of suitable climate, topography, etc., in North and South America. A variety of disease surveys in lower animals are currently being carried out in the United States; the agents studied in these surveys should include the VEE virus.

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