PUBLIC HEALTH MONOGRAPH NO. 23

Biological Factors In the Transmission Of Arthropod-Borne Virus Encephalitides

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Biological Factors In the Transmission Of American Arthropod-Borne Virus Encephalitides

A Summary

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Contents

Introduction

The aim of this paper is to present a selected bibliography with annotations, for the use and information of investigators and to furnish pertinent tabular summaries for those who are actively engaged in studies on the epidemic encephalitides in the Americas. The general goal of the workers in this field has been clarification of the problem of how the etiological agents are maintained and transmitted in nature, with the hope that practical means of dealing with these diseases may be found. This review stresses the entomological phases of encephalitis transmission.

The text of the review reflects principally the winnowing of data for inclusion in a tabular summary. Subject literature has been covered through April 1952. Some indication of the content of each bibliographic item (but not relative values) has been given. However, in a work of this magnitude, there will remain errors involving judgment, interpretation, and other variables, as well as transcription difficulties. Change of concepts in this rapidly developing field must be considered, for in many cases the ideas expressed in early works are no longer tenable and, in some instances, unpublished data may change the emphasis on, or even refute, a concept previously delineated. In this regard, the author has adhered to published materials, with but little exception.

The published data on arthropod-borne neurotropic viruses of Central and South America are limited and are briefly summarized for comparative purposes. The following types of encephalitis or encephalitis-producing viruses occur there: Argentine equine encephalomyelitis (*176*, *253*) in which the virus is identical (*329*) to that of western equine encephalomyelitis (WEE [1]); eastern equine encephalomyelitis (EEE), now distributed in Panama, Brazil, Mexico, Cuba, and the Dominican Republic; Ilheus encephalitis (*218*) from Brazil, a mosquito-borne human infection; *Anopheles* A, *Anopheles* B, and *Wyeomyia* encephalitis (*328*) from Colombia, known as mosquito-borne viruses capable of producing these diseases in laboratory animals; *Anopheles* I, Sabenthine I, and an unnamed virus from *Psorophora ferox* infective for laboratory animals, also from Colombian wilds (*64*); and Venezuelan equine encephalomyelitis (VEE) (*21*, *42*, *116*, *215*, *216*, *308*), a disease of man and equines, now established in Trinidad, Venezuela, Colombia, and Ecuador. VEE is marked by explosive outbreaks among equines and appears to be transmitted by mosquitoes in nature and possibly by dust under laboratory conditions. For published details on VEE, refer to the bibliography maintained at the Camp Detrick Technical Library, Frederick, Md. (*2*). There is a paucity of data on the vector problem related to these Central and South American virus diseases (*308*). The arthropod-carried viruses reported as being capable of causing encephalitis found in the Western Hemisphere are these: EEE, WEE, SLE, Hammon-Reeves or California,[2] Ilheus, *Anopheles* A, *Anopheles* B, *Wyeomyia*, *Anopheles* I, Sabenthine I, the unnamed virus from *P. ferox*, and VEE.[3]

There have been but few attempts to classify the confusing series of diseases manifested as encephalitis (*28*, *32*, *34*, *101*, *131*, *412*). Ayres and Feemster (*32*) in 1949 divided these diseases

[1] Symbols used in the literature: WEE (western equine encephalomyelitis); EEE (eastern equine encephalomyelitis); SLE (St. Louis encephalitis); VEE (Venezuelan equine encephalomyelitis); type A (von Economo's disease or lethargic encephalitis); type B (Japanese encephalitis); type C (St. Louis encephalitis).

[2] The California virus strain (BFS-91) was isolated from *Aedes dorsalis* from Kern County, 1943 (*147*).

[3] References on certain foreign neurotropic viruses are presented for comparative purposes: VEE (*21*, *42*, *116*, *215*, *216*, *308*); *Japanese B encephalitis* (*65*, *192*, *307*); Russian tick-borne encephalitis (*75*); Australian X disease (*76*); Australian encephalitis (*16*); Ilheus virus (*218*); West Nile virus (*292*, *375*); *Anopheles* A and B, and *Wyeomyia* viruses (*328*), Argentine equine encephalomyelitis (*253*); and louping ill (*412*).

into three groups: "Virus encephalitides caused by familiar viruses not ordinarily encephalitogenic; suspected, primary virus infections; and primary virus infections."

In the first group, the following are usually listed: herpes simplex, measles, lymphogranuloma venereum, mumps, and infectious mononucleosis. Postinfective encephalitis as an aftermath of infectious hepatitis, smallpox, vaccinia, chickenpox, influenza, mumps, and measles also belong to this group, along with postvaccinal encephalitis due to vaccination against rabies and smallpox.

In the second group—suspected primary virus infections—belong types in which antibodies have been found in the blood of man and the viruses are shown to be neurotropic in laboratory induced disease. In this category are the following virus diseases: West Nile (*375*), Bwamba fever (*376*), Semliki Forest (*373*), Bunyamwera (*377*), Ilheus (*218*) and California (*147, 149*) in which the syndrome in man has not been adequately described. Von Economo's disease (encephalitis lethargica) and acute disseminated encephalomyelitis also are suspected of being caused by primary neurotropic viruses. Due to the gradual disappearance of von Economo's disease in the United States between 1920 and 1930, it is not at present considered important epidemiologically.

In the third group belong the primary virus encephalitides, which may be divided into the summer, or epidemic, forms: St. Louis encephalitis; eastern, western, and Venezuelan equine encephalomyelitis (*216*); Japanese B encephalitis (*412*); Russian Far East (tick-borne, or spring-summer) encephalitis (*75*); Australian X disease (*76, 287*); and into the endemic viruses: acute hemorrhagic meningoencephalomyelitis, or leukoencephalitis; lymphocytic choriomeningitis; pseudolymphocytic choriomeningitis; swineherd's disease (*91*); louping ill (*412*); acute encephalitis due to rabies; and Sabin B virus. The chief medical problem in North America appears to lie with the epidemic encephalitides (EEE, WEE, and SLE), in which the mortality ranges from 5 to 60 percent.

The references listed constitute a bibliography in the sense that, in the author's opinion, all important papers relating entomology to encephalitis published prior to 1952 have been included. The literature is vast and widely scattered in many fields of science. The *Quarterly Cumulative Index Medicus* (American Medical Association) lists thousands of references, garnered from international sources before 1930, which pertain to the purely human clinical manifestations of alleged encephalitis. Since 1930, references to the particular diseases WEE, EEE, and SLE may be found generally concentrated in the sources discussed.

Information is scattered throughout the standard abstracting journals and reference indexes (*Biological Abstracts*, *Review of Applied Entomology—Series B*, and *Bibliography of Agriculture*, *Quarterly Cumulative Index Medicus*, and *Index of American Economic Entomology* by Banks, Colcord, and Hawes). The standard texts and reference books, such as those of Rivers (*327*), Van Rooyen and Rhodes (*412*), Bates (*36*), Smith (*363*), and Neal (*280*), are cited.

The various State agricultural bulletins contain data generally designated for lay consumption and pertain mostly to the care of horses suffering with these diseases. The annual reports of the U. S. Bureau of Animal Industry constitute a source from which data on the prevalence of WEE and EEE in the United States may be obtained. Veterinary journals (*American Journal of Veterinary Research*, *North American Veterinarian*, and *Journal of American Veterinary Medical Association*) inform the veterinary profession of new developments in the field and contain many important reviews.

With respect to encephalitis in man, some reviews are to be found in the *New England Journal of Medicine*. Technical studies on clinical cases, epidemiological summaries, and editorials designed to clarify a confused picture of disease for the practicing physician are to be found in the *Journal of the American Medical Association*. Various State medical journals contain clinical case reports and invitation-type papers which are written for uninformed practicing members of the profession. Technical studies on the viruses are to be found in the *Journal of Immunology*, *Archives of Pathology*, and similar publications. Experiments involving laboratory transmission of these diseases have been described most often in the *Journal of Experimental Medicine*, *Proceedings of the Society of Experimental Biology and*

Medicine, *Journal of Infectious Diseases*, and *American Journal of Tropical Medicine*. In general, epidemiological studies have been published in the *Canadian Journal of Comparative Medicine*, *Canadian Journal of Public Health*, *Public Health Reports*, *Public Health Service Bulletins*, *American Journal of Hygiene*, *American Journal of Public Health*, and *Proceedings of the California Mosquito Control Association*. Numerous preliminary reports are to be found in *Science*. The Matheson Commission of New York has accumulated and distributed data on the epidemiology of human encephalitis, and the Hooper Foundation of California has made extensive contributions in the general field of epidemiology of the diseases (principally WEE and SLE) in the United States.

Useful general reviews are the following: Ayres and Feemster, 1949 (*32*); Casey, 1942 (*69*); Cockburn et al., 1951 (*79*); Davis, 1940 (*87*); Dingle, 1941 (*89*); Feemster and Getting, 1941 (*99*); Fothergill, 1940 (*101*); Geiger et al., 1922 (*108*); Giltner and Shahan, 1942 and 1936 (*115*, *114*); Hammon et al., 1948, 1948, 1945, 1945, 1945, 1942 (*141*, *142*, *160*, *138*, *147*, *152*, respectively); Manzelli, 1948 (*235*); Matheson Commission, 1939 (*426*); Meyer, 1933 and 1933 (*249*, *250*); Milzer, 1950 (*259*); Neal, 1942, 1934, 1928 (*280*, *279*, *277*, respectively); Price, 1950 (*296*); Reeves, 1941 (*305*); Schoening, 1940 (*337*); Shahan and Giltner, 1945 (*348*); Sulkin, 1949 (*382*); Udall, 1913 (*409*); Webster, 1941 (*417*); Wright, 1927 (*429*), and Zinsser, 1928 (*432*). Certain papers are given minor consideration in this review because either they do not contain results of original tests or the information is of a generalized nature (*7*, *10*, *14*, *17*, *19*, *43–45*, *54*, *55*, *60*, *63*, *67*, *96*, *130*, *134*, *140*, *143*, *201*, *230*, *237*, *244*, *274*, *295–297*, *303*, *306*, *309*, *325*, *326*, *334*, *339*, *347*, *378*, *387*, *400*, *404*, *427*). In the literature, the term "encephalitis" is used somewhat loosely to cover both encephalitis involving virus infection in the brain proper and encephalomyelitis which is centered in the brain and spinal cord. Encephalitis is popularly known as sleeping sickness or brain fever.

Historical Notations

Four types of encephalitis (literally, brain inflammation) have appeared in epidemic form in the United States within the last 35 years:[4] von Economo's disease (encephalitis lethargica or Japanese A encephalitis), eastern and western equine encephalomyelitis, and St. Louis encephalitis.[5] All of these forms affect the central nervous system.

Encephalitis Lethargica

Although von Economo's encephalitis is an ancient disease (*232*, *362*, *414*), in modern times it has been best known from about 1915, when it was studied in France by Cruchet et al. (*85*). It is clinically manifested by somnolence, oculomotor palsy, pyrexia, and signs of cerebromeningeal disturbance. This disease of unknown etiology was established in epidemic form in New York, N. Y., and in Winnipeg, Canada, in 1919–20, but by 1926 it had begun to subside in North America and the world over. Since then, the disease has become relatively rare (*173*, *276–280*), and possibly the cases seen now are of different etiology. Arthropods have not been suspected of transmitting this essentially winter-season disease.

St. Louis Encephalitis

Briefly, the history of SLE is as follows: The first cases of the disease to become known as St. Louis encephalitis occurred in Paris, Ill., in 1932, although there is evidence that it has long been endemic in the area (*79*). Cases

[4] See table 7, p. 17.

[5] In addition to SLE, WEE, and EEE viruses, there is the California virus named by Hammon and Reeves which has not been studied in man (*147*, *149*).

occurred in St. Louis County, Mo., and in surrounding areas in a wide radius during the summer and fall of 1933 in epidemic fashion (*79, 171, 294*). A minor outbreak occurred in Toledo, Ohio, in 1934, and neutralizing antibodies were later recorded for the residents of New York and Pennsylvania. The disease subsided with no true cases being reported for 1935–36. However, it reappeared in epidemic form in the summer of 1937 in St. Louis County, Mo. (*61, 69–70, 121*). Several hundred cases developed, and the disease has since become established endemically in the United States, having occurred notably in California and Washington. Ten cases in 1938 failed to show SLE antibodies (*366*), but about one-third of 66 cases during 1939–44 gave serologic evidence of SLE antibodies (*50*). Results from a serum survey in 1943–44 (*371*) indicate that there were fewer persons with SLE antibodies who came to California and Washington after 1937 as compared with persons with SLE antibodies resident there during 1933–37. Some of the details of the continental distribution of SLE in man may be found in the following papers: Arizona, (*238*); California (*159, 179, 184*); Colorado (*22, 293*); Minnesota, 1941 (*92*); Missouri, 1933 (*272*); Nebraska, 1941 (*431*); New York (*57, 419*); North Dakota (*56*); Oklahoma (*315*); Utah (*26*); and Washington (*129, 144*). There is evidence that SLE occurs as a human disease in Africa (*372, 374, 375*). Some of the reports of virus isolations from man are cited (*51, 61, 225, 238, 273*).

St. Louis encephalitis is not generally thought of as being "equine" in nature, although it does occur in horses (*1, 11, 84, 144, 153, 293*) as a subclinical infection, and under laboratory conditions it may induce equine deaths (*351*). The susceptibility of mules to SLE virus infection also has been confirmed (*18, 84*).

Western Equine Encephalomyelitis

Zwick and Seifried (*433*), working on European equine encephalomyelitis in 1924, determined that the etiological agent of that disease was a filtrable virus. In 1932, Meyer (*248*) recorded the possibility of human infection following infection of horses with WEE virus and subsequently described the first cases in man. Howitt (*178*) isolated WEE virus from the brain of man in 1938 and from human serum in 1939 (*180*). Numerous human cases have been reported from California. The disease has been found in Minnesota, 1938; Arizona, 1941; North Dakota, 1938 and 1941; South Dakota, 1941; Nebraska, 1941; Montana, 1947; Texas, 1944; Manitoba, Alberta, and Saskatchewan, Canada, 1941; and Manitoba, Canada, 1947. The virus of Argentine equine encephalomyelitis appears to be identical with that of WEE (*275, 353*). A few isolations of WEE virus have been made from human materials (*67, 82, 128, 178, 223, 239*).

Eastern Equine Encephalomyelitis

Human infection with EEE virus first was recognized as a nosologic entity with the isolation of the agent by Fothergill et al. (*103*) and Webster and Wright (*422*) in Massachusetts in 1938. This development followed the original isolation of EEE virus from infected horses by Giltner and Shahan in 1933 (*112*). In man the disease followed a fulminating course resulting in fatality of 74 percent of the 34 persons affected in the first outbreak (*33, 425*) and produced somewhat similar results in a later Louisiana outbreak (*257, 282*). There has been only scattered evidence of human infection since 1938: Indiana, 1939; Louisiana, 1947 (*169, 257*); Tennessee, 1947; and Texas, 1941–42 (*52, 117, 136*). A few virus isolations from man have been reported (*103, 257, 282, 338*). The history and present public health significance of EEE has been summarized recently by Beadle [6] who noted that EEE virus had been demonstrated in warm-blooded forms (man, horses, and birds) in all of the States bordering the Atlantic Ocean and the Gulf of Mexico plus Michigan, Missouri, Arkansas, and Tennessee.

[6] Beadle, L. D.: Eastern equine encephalitis in the United States. Mosq. News 12 (2): 102–107 (1952).

Laboratory and Field Techniques and Results

The difficulties encountered in inducing virus infection in warm-blooded animals or in transmitting these viruses by means of arthropod vectors oftentimes have been overcome through sheer ingenuity. Of special interest are the arthropod-vector techniques employed by early experimenters such as Meyer et al. (*252*), Simmons et al. (*355*), Knowlton and Rowe (*213*), and Kelser (*202*). Many descriptions of the presently used methods are available (*6*, *71*, *72*, *155*, *157*, *341*, *421*).

Arthropods of many species have been ground up and injected into laboratory animals in an effort to isolate the viruses WEE, EEE, and SLE from nature (*18*, *105*, *114*, *124*, *212*, *316*, *368*). The feces of mice (*24*) and assassin bugs (*124*) have likewise been used. Mitamura et al. (*260*) used groundup mosquito eggs in an effort to show transovarial passage of Japanese B virus. The fresh brain tissue of man, taken at autopsy, has been ground and injected into horses (*338*), producing either fatal or mild cases of encephalitis. Brain tissue has also been injected into mice (*418*) with positive results.

Reeves et al. have presented special methods employed in the attempt to transmit SLE virus directly by the bite of *Culex pipiens* (*314*). The viremia of such agents is short lived, relatively speaking, and chance has been an important factor in working with them. In Trinidad, Gilyard (*116*), working with one of the longer-lived viremias, noticed that *Mansonia titillans* was predominant among the mosquitoes taken. Gilyard trapped six female mosquitoes feeding on the back of an encephalitic donkey, and by placing them on a normal-appearing donkey he obtained direct evidence of virus transmission by arthropod biting in that epidemic of Venezuelan equine encephalomyelitis.

Intranasal instillation of virus suspension has been commonly used, especially in early tests, in trying to demonstrate droplet infection via the upper respiratory tract of test animals (*175*, *302*, *388*, *411*, *413*). Several authors have resorted to the use of the embryonic chorioallantoic membrane in order to show arthropod transmission in laboratory animals (*342*, *367*, *368*, *370*, *371*).

Isolation of Virus

The technique of Hammon et al. (*155*) for virus isolation from field-caught arthropods has become a standard; by this technique, specimens are identified under chloroform anesthesia, hermetically sealed by flame in shell vials, packed in dry ice, and held for later thawing and grinding of the specimens preparatory to injection into test animals, for which mice are generally suitable. After the mice have manifested encephalitic symptoms, their brains are removed aseptically, triturated, and passed to other mice. Upon establishment of a strain of virus, it is identified by using the neutralization test.

Many specimens of the same species of arthropod (especially mosquitoes) taken from the same place are often pooled for a single injection. Obviously, this technique demands proper speciation when possible. One notes that in preliminary studies Semliki Forest virus (*373*) was recovered from a taxonomically confused group of *Aedes* sp., and that Bunyamwera virus (*377*) was isolated from 15 specimens of a pool of 4,114 mosquitoes belonging to 14 species of *Aedes*. In this country, isolations of virus have been made from pools of arthropods containing different species (*187*, *316*). Arthropod collecting and handling under field conditions have been detailed in the following works: *115*, *213*, *240*, *293*, and *312*.

All of the life-cycle stages of certain candidate arthropod vectors (especially ticks and mites) have been utilized in attempts to show transovarial transmission of these viruses (*368*, *369*, *390*, *393*). Old and young viremic mice and infected ticks have been successfully manipulated to show transmission of SLE (*47*).

Brodie (*58*), working with SLE, was unable to infect normal mice orally with contaminated food or by intragastric injection, using laparot-

omy to rule out intraperitoneal infection. Details on an unusual laboratory method of virus transmission by one mouse eating another have been given by Harford et al. (*164*, *165*). Hogs were shown to be resistant to the feeding of several pints of WEE-virus-laden chick embryo materials (*241*).

Davis (*87*) made comparative studies on the ability of various mosquitoes to infect birds with EEE by biting. He showed that in a 25-day life span of an individual *Aedes triseriatus*, this mosquito succeeded in transmitting the virus to three different guinea pigs and to a cowbird by a bloodless puncture. TenBroeck and Merrill used the unusual approach of raising the "titer" of WEE-infected *Aedes aegypti* by continued serial feedings of normal mosquitoes on crushed infected mosquitoes (*398*). Growth of WEE has been accomplished by using the tissues and organs of *A. aegypti* in vitro; and it was found that the midgut of full-grown larvae and the ovaries of newly emerged adults best supported virus growth (*405*). Virus is thought to be uniformly distributed in the parts of infective *A. aegypti* (*245*). Initial feeding of arthropods on artifically infected animal hosts (via virus injection) or on virus suspensions of various kinds has, of necessity, been the rule in many laboratory tests. (References *131*, *132*, *145*, *146*, *245*, *260*, *379*, *399* depict the use of virus in blood, in brain suspensions, or in other media as a source of infective agent.)

Arthropods have been test-fed on a large number of warm-blooded candidate virus reservoirs (table 6). Of all the common routes by which infection may be given to candidate hosts, the intracranial or intracerebral injection and the dermal route have been most widely utilized. Comparative immunological studies on domestic encephalitis viruses (EEE, WEE, and SLE) and similar studies on those of foreign origin are to be found in these papers: *176*, *177*, *215*, *217*, *228*, *253*, *288*, *302*, *346*, *365*, *416*, *420*. An analysis of routine laboratory methods has been prepared by Milzer (*259*).

Laboratory Infections

Laboratory virus infections in man induced by accident or by other means serve as a source of valuable information when the cases can be adequately studied. Sulkin and Pike have furnished general review up to 1949 of viral infections contracted in the laboratory (*384*). Casals et al. summarized two such infections with Venezuelan equine encephalomyelitis which were notable for the isolation of that virus from the nasopharynx and blood of these nonfatal cases (*68*). Lennette and Koprowski (*227*) were able to study eight patients with the same disease, which apparently was dustborne. Two of the four accidental WEE cases in technicians have resulted in death (*104*, *119*, *170*). The case of WEE followed by Gold and Hampil (*119*) is especially interesting in the description of the successful use of antiencephalomyelitic immune horse serum administered in large volumes and at frequent intervals. This method is without value after CNS symptoms occur. One worker who handled EEE for 6 years and enjoyed apparent good health developed neutralizing antibodies in his serum (*284*). To date there seem to have been no cases of laboratory infection with SLE.

Epidemiology

Man is not considered to be a reservoir of encephalitis virus and may be but an accidental host (*141*). Furthermore, although devastating epizootics have occurred in the United States and in Canada among the equine species, these animals are not considered as important sources of virus agent. Disease in horses and in humans (*21*) serves as an epidemiological indicator of the presence of infection in certain areas such as California (*150*) and Washington (*144*). It is sometimes stated that the reporting of human cases of epidemic encephalitides has been more carefully done in the Western States than in the States east of the

Mississippi River. Since 1930, a total of 14,119 human cases has been reported [7] in the 22 Western States, the lowest number (226 cases) in 1948 and the highest number (3,156 cases) in 1941. The year 1950 had the highest number (554 cases) since 1941, and of these infections 333 were from California. The National Office of Vital Statistics reports a total of 27,748 human cases for the period 1926–50 in all States (the lowest number, 702, in 1932; the highest, 3,516, in 1941; and 1,135 cases in 1950).

Equine Encephalomyelitis

The term "equine encephalomyelitis" was first applied to this disease entity by C. H. Stange in November 1912 when he spoke before the Iowa State Veterinary Medical Association (*12*). At this time the disease in horses was poorly understood and was variously known as horse plague, Borna's disease, botulism, forage poisoning, and sleeping sickness (*220*). Zwick and Seifried, working on European materials, in 1924 (*433*) disclosed that the equine disease was caused by a filtrable virus; this preceded the classic work of a similar nature by Meyer and co-workers in the United States (*252*). Naturally, the horse is the animal which has been the most studied clinically. Although all of the papers cited (*17, 86, 154, 240, 275*) have interesting descriptions of equine disease, the early works of Records and Vawter (*299–303*) are noteworthy for the inclusion of a series of photographs of animals with equine encephalomyelitis in progressive stages. Motion pictures were taken of horses with symptoms of the disease in the Montana outbreak of 1936 (*275*).

Equine epidemics (presumably involving EEE and WEE) have been recorded for the United States in 1847, 1850, 1867, 1882, 1897, 1912, 1919, 1920, 1921, 1923–28, and 1930–31 (*220, 249, 267*). Morbidity and mortality data for these early epidemics are relatively scarce (*249*). The 1912 epidemic seems to have been rather widely distributed, covering 17 States, with an estimated loss of 35,000 horses and mules in Kansas and Nebraska (*249*) alone. Meyer early thought of these first epidemics as being cyclic in nature since the peaks seemed to come at 10- to 15-year intervals. Since 1936, yearly reports by Miller (*255, 256*), Mohler (*263, 265, 266, 268–271*) and Simms (*356–361*) from the Bureau of Animal Industry (U. S. Department of Agriculture) have presented a continuous picture of the disease in horses. The data are accumulated through the cooperation of local veterinarians, livestock officials, and Bureau inspectors and are often based on questionnaires. A 15-year summary (*361*) up to 1951 shows the following peak years, with numbers of reported cases: 1935 (23,512); 1937 (173,889); 1938 (184,662, the highest number in 15 years); 1940 (16,941); 1941 (36,872); and 1944 (19,590). Since 1944, incidence has gradually dropped to the lowest recorded figure of 762 for 1951.

The number of deaths from equine encephalomyelitis during the period 1935–51 ranged from 274 (1951) to 8,210 (1941), with a total of 33,563 equine deaths recorded for the 13-year period (1939–51). Equine deaths are not recorded for the years 1935 through 1938 in the May 1951 report by Simms (*361*). The mortality per 100 affected animals has ranged from 21 in 1938 (the peak year of the period 1935–50) to 60 in 1949 (with only 4,037 cases), with an average mortality of 33.2 since 1938.[8] On the basis of cursory analysis, there is apparent indication of a possible spread and shifting of the disease (lumping the incidence of WEE and EEE) if one considers the number and location of the States affected either epidemically or endemically, as indicated by the Bureau of Animal Industry reports, noting cases in 17 States in 1912, and 28, 17, 30, 39, 42, 38, 33, 35, 34, 33, 33, 37, 33, 35, 37, 33, and 28 States involved for the years 1935 through 1951, respectively.

Geographic Distribution

There is no adequate evidence that these diseases have spread geographically from a central focus in North America. As an example of the distribution, 96 percent of all cases were

[7] It should be pointed out that this rather large number of cases primarily represents the reporting of "infectious encephalitis" and not "arthropod-borne virus encephalitides" alone, for the number of laboratory confirmations of the latter cases is a small proportion of the total figure.

[8] Combination of mortality rates for WEE and EEE in the horse is unfortunate since it is sometimes stated that the rate for WEE usually averages 20–40 percent, whereas the rate for EEE averages 80–90 percent.

located west of the Mississippi River in 22 States in 1940; whereas in 1950, cases were rather evenly and lightly distributed north of the Ohio River and west of the Mississippi River, with the highest concentrations (5 or more cases per 1,000 equines) in the extreme southern sectors of Oregon and in parts of California. In general, California, especially the central regions, appears to have been the 1950 focal point of disease distribution. During the epizootic period of 1951, California, Nebraska, and Missouri had the highest indexes. The disease has remained rather statically distributed since the peak year of 1938, while the lowering of the index has been gradual. This has been coupled with a decreasing equine population in the United States—from 15,245,000 (1937) and 9,130,000 (1948) to a figure of 7,463,000 in 1951. The range in the number of States affected by the disease has been 17 (1936) to 41 (1939); in the latter case, all States except Mississippi, Tennessee, South Carolina, West Virginia, Pennsylvania, New York, and New Hampshire had reported the equine disease. At this writing, equine disease has not been reported from Tennessee, West Virginia, and Pennsylvania. The following Provinces in Canada have reported equine infections: Ontario, Manitoba, and Saskatchewan, where the first case was noted in 1935 (*107*). Actual virus isolations of WEE and EEE from equines of 29 States up to 1951 are presented in table 5.

These equine diseases probably are widely distributed in many foreign countries, as evidenced by the following references: EEE and WEE in Canada (*341*, *348*); EEE in Panama (*95*, *196*, *199*, *380*), in Brazil (*95*, *226*), in Mexico (*95*, *348*), in Cuba (*95*), in the Dominican Republic (*3*, *94*, *95*), and in the Philippine Islands (*231*); WEE in Argentina (*253*, *176*); while equine encephalomyelitis is assumed to have been reported for both Hawaii and Puerto Rico (*361*).

Many data representing the various facets of epidemiology have been gathered in numerous surveys of these human and animal diseases in North America, of which the following list represents the major efforts for each: SLE (*18*, *53*, *69*, *129*, *137*, *144*, *149*, *150*, *152*, *154*, *156*, *159*, *179*, *182*, *184*, *221–224*, *229*, *242*, *382*); WEE (*66*, *77*, *83*, *90*, *92*, *106*, *137*, *149*, *150*, *152*, *154*, *156*, *158*, *160*, *167*, *179*, *181*, *184*, *291*, *299*, *315*, *430*); EEE (*87*, *94*, *98*, *99*, *110*, *112*, *158*, *189*, *206*). With respect to field methods, the questionnaires designed for use in the famous Missouri SLE outbreak and the questionnaire supplied observers in equine outbreaks (*249*, *263*) are of interest. Probably the most complete study of arthropod vectors of EEE outbreaks was that of Feemster and Getting (*99*), in which the data of an extensive survey have been presented. Analysis of the recent (1948–49) epidemic of EEE in the Dominican Republic, by Eklund and co-workers (*94*, *95*), was made by use of the most advanced techniques.

Climate and Seasonal Appearance

Climate and the seasonal appearance of these diseases (WEE, EEE, and SLE) have received considerable attention from epidemiologists. Since all but one (Russian tick-borne encephalitis) of the epidemic encephalitides are held to be mosquito-borne, and since they have occurred epidemically principally in temperate regions, the distribution has been primarily in summer and fall months. The Bureau of Animal Industry reports on horses have selected three seasonal periods (pre-epizootic, epizootic, and postepizootic) as useful indicators. As an example, these periods, with respective months and number of cases per month, are presented for 1950 (*361*): pre-epizootic—January (7), February (6), March (3), April (11), and May (41); epizootic—June (94), July (153), August (218), September (303), October (141); postepizootic—November (39) and December (7).

Many of the equine outbreaks in the United States have appeared under hot, dry conditions. Such was the case in the San Joaquin Valley in 1931, where the number of cases rose when the temperature ranged between 90° and 100° F. At that time it was tentatively thought that this condition brought out inapparent cases (*249*). High humidity was recorded for the disastrous horse epidemic of 1912 in Kansas and in more recent outbreaks in Saskatchewan, Canada. Records and Vawter (*300*) reported three infected horses in Nevada in November. These animals had been moved in from a high mountain ranch to another corral to join a herd of horses in which there had been no apparent infection for 15 months, and with-

in 8 days, each of the three animals developed a fulminating viremia. The authors felt that, in general, disease transmission via mosquitoes was unlikely since several "localized outbreaks with a high mortality have occurred in the winter months."

Lambert et al. (*219*) found that young horses were more susceptible to WEE than older ones, but that sex differences did not show in a statistical analysis of a Miles City, Mont., outbreak in 1939. Low indexes of clinical cases in animals over 2 years old may in large part be due to immunity developed by clinical or, more probably, nonclinical infection at an early age. At present, most infections appear in horses under 2 years of age. The fact that mice (*283*) and chickens (*262*, *385*) are more susceptible to viruses when young forms the basis for several laboratory procedures. Young birds have been studied as sources of these viruses in nature (*379*). Chicks may serve as a short-term source of WEE virus for mosquitoes (*148*).

Certain unusual findings in equine epidemics are included here for consideration. Fulton (*107*) noted that Saskatchewan outbreaks of WEE occurred in areas known to be very dry for months and to be without mosquitoes. Meyer also commented on the same thing early in the history of the disease (*251*). Early studies on horse botulism showed the disease (which included "sleeping sickness") to be most prevalent in the fall and winter, which is a seasonal distribution not generally found at present in WEE and EEE. In a notable virus isolation of EEE from a Florida horse during the middle of January 1939, the wintertime occurrence was tentatively attributed to the probable presence of mosquitoes (*15*, *285*). One horse case of WEE occurred in the center of an area not completely treated for the insect (*163*).

Human Encephalitis

An interesting human case of WEE (*324*) occurred in the center of Chicago, and allegedly careful checking showed no known contact with arthropods. One wonders at the western distribution of SLE virus in the United States and its apparent absence in Canada, as reflected in reporting. Certain manifestations of these viruses which appear unusual at present have been reported, such as the development of a virus which caused lymphocytic choriomeningitis in monkeys and mice upon the injection of a virus suspension taken from a patient dying with SLE (*31*); the suggested relationship existing between SLE and epidemic keratoconjunctivitis (*74*, *332*); certain changes in viruses (*406*), especially the isolation of a mixture of viruses from which both SLE and WEE were obtained (*162*); and the discovery of an agent in normal mouse urine which neutralized large amounts of SLE virus (*286*).

In recent years SLE has become increasingly more important in California. At present, two established endemic foci of WEE are Weld County, Colo., and Kern County, Calif. Moreover, new viruses have been discovered, such as the California virus (*159*).

The age distribution of human cases of virus encephalitis has been considered, especially the distribution of SLE (*50*). In general, age distribution varies with the immunity and type of exposure of a population. SLE was shown to have a higher case index among older groups but no preference for sex or color.

Transmission of Virus

Kelser's researches (*193*) on the direct transmission of WEE virus through the bloodsucking activity of an arthropod are classic in the history of the epidemic encephalitides. Kelser used *A. aegypti* of Philippine Islands origin and WEE virus from the brain of a North Dakota horse in the first laboratory transmission of encephalomyelitis. He injected virus suspensions into three guinea pigs. Mosquitoes were initially fed on the three guinea pigs at 48, 72, 96, 120, and 144 hours following injection, a period which covers the febrile stages of the disease in guinea pigs. Each lot of *Aedes* was then test fed on normal guinea pigs 6 days from the time of initial feeding, an interval thought of as the incubation period. All guinea pigs died from encephalitis from each of the mosquito test groups except the last one (144 hours). Some of the mosquitoes were infective up to 18 days after initial feeding. A horse which also was infected by the above method of direct transmission by arthropod bite died with encephalomyelitis. TenBroeck and Merrill learned later (*399*) that *Aedes* sp. must

Table 1. Results of attempted virus isolations from nature (arthropods)

Arthropods studied	Western equine encephalomyelitis	St. Louis encephalitis	Eastern equine encephalomyelitis
Aedes aegypti	−315	−315	
Aedes campestris	−151	−151	
Aedes cinereus	−151, 156	−151, 156	
Aedes dorsalis	+147, 149, 159; −151, 156, 424	+147, 149; −151, 156	
Aedes increpitus	−151, 156	−151, 156	
Aedes lateralis (sticticus)	−151, 156	−151	
Aedes nigromaculis	−137, 149, 156, 160, 315, 424	−137, 315	
Aedes scapularis	−158	−158	−94, 158
Aedes sollicitans	−158	−158	−158
Aedes taeniorhynchus	−158	−158	−94, 158
Aedes triseriatus	−160		
Aedes trivittatus	−160		
Aedes vexans	−124, 137, 149, 151, 156, 158, 160, 315, 424	−137, 149, 151, 158, 315	−158
Anopheles albimanus			−94
Anopheles crucians	−158	−158	−94, 158
Anopheles freeborni	+137, 147; −156, 151	−137, 151, 156	
Anopheles pseudopunctipennis	−158	−158	−158
Anopheles punctipennis	−137, 156, 160	−137	
Anopheles quadrimaculatus	−158	−158	−158
Culex bahamensis			−94
Culex coronator	−158	−158	−158
Culex erraticus	−158, 315	−158, 315	−158
Culex pipiens	+137, 147; −151, 156, 160	+137; −151, 156	−98
Culex quinquefasciatus	−147, 149, 158, 315	−149, 158, 315	−158
Culex restuans (territans)	+281; −124, 160		
Culex salinarius	−158, 160, 315	−158, 315	−158
Culex stigmatosoma	+147, 159; −149	−147, 149, 159	
Culex tarsalis	+4, 8, 23, 137, 147, 149, 151, 156, 159, 160, 211, 424; −124, 158, 159, 311, 315	+132, 137, 151, 156; −158, 315	−158
Culex thriambus	−149	−149	
Culiseta incidens	−137, 151, 156	−137, 151, 156	
Culiseta inornata	+137, 147; −149, 151, 156, 315	−137, 149, 151, 156, 315	
Culiseta melanura			+26, 73
Deinocerites cancer			−94
Deinocerites spanius	−158	−158	−158
Mansonia perturbans			+188, 206

be fed on virus suspensions (or on animals injected with virus suspensions) of high titer in order to secure uniform results.

Very few isolations of EEE have been made from arthropods in nature (table 1), although mosquitoes (as well as horses) were suspected as factors in disease transmission in early epidemics (*425*). Chamberlain and co-workers made an isolation from the mosquito *Culiseta melanura* in Louisiana (*73*). Before this, the virus had been detected in Tennessee in chicken mites and chicken lice (*187*), and in Georgia in a mosquito, *Mansonia perturbans* (*188, 206*).

The present record on WEE virus isolations (as based on initial dates) from the important vector species *Culex tarsalis* in the United States is as follows: Washington, 1941; California, 1943; Nebraska, 1943; Montana, 1944; and North Dakota, 1948. Through 1950, 144 isolations of WEE and 14 isolations of SLE virus have been made from this important mosquito. Thompson and co-workers have recently reported on the isolation of WEE virus from *A. dorsalis* of midwestern United States for the first time.[9]

One of the main purposes of this survey is to present tabulations on intermediate and definitive hosts. Studies are summarized in tables 1 through 6, which are complete through 1951. In the use of these tabulations it must be re-

[9] Thompson, G. A., Howitt, B. F., Gorrie, R., and Cockburn, T. A.: Encephalitis in Midwest. VI. Western equine encephalomyelitis virus isolated from *Aedes dorsalis* Meigen. Proc. Soc. Exper. Biol. & Med. 78: 289–290 (1951).

Table 1. Results of attempted virus isolations from nature (arthropods)—Continued

Arthropods studied	Western equine encephalomyelitis	St. Louis encephalitis	Eastern equine encephalomyelitis
Mansonia titillans	−158	−158	−158
Psorophora ciliata	−158, 160, 315	−160, 315	−158
Psorophora confinnis	−315	−315	
Psorophora cyanescens	−158, 315	−158, 315	−158
Psorophora discolor	−158	−158	−158
Psorophora pygmaea			−94
Psorophora signipennis	−158, 160, 315	−160, 315	−158
Bdella sp	−424		
Dermanyssus americanus	+254, 316;[1] −311	−316	
Dermanyssus gallinae	+381; −311, 315, 424	+367, 368; −315	+187
Bdellonyssus bursa	+382		
Bdellonyssus sylviarum [2]	+162, 316, 424; −311	+4, 162	
Pteronyssus sp	−424		
Argas persicus	−149	−149	
Dermacentor americanus	−316		
Dermacentor andersoni	−125, 127	−149	
Haemaphysalis leporis	−149	−149	
Ixodes sp	−156	−156	
Ornithodoros turicata	−149	−149	
Ground squirrel fleas	−149	−149	
Rabbit fleas	−149	−149	
Assassin bugs	−127		
Triatoma sanguisuga	+124, 209		
Cimex lectularius	−160, 156	−156	
Menopon pallidum			+187
Eomenacanthus stramineus			
Ground squirrel lice	−149	−149	
Ceratopogoniids	−156	−156	
Haematobia serrata	−315	−315	
Hippelates pusio	−156	−156	
Siphona irritans	−156	−156	
Musca domestica	−156	−156	
Simulium sp	−156	−156	
Stomoxys calcitrans	−124, 156	−156	
Stomoxys sp	−127		
Tabanus sp	−156	−156	

[1] Original collection was a mixture of *D. americanus* and *B. sylviarum*. [2] Also listed as *Fonsecaonyssus sylviarum*.

NOTE: + or − preceding bibliographic number indicates positive or negative results during a particular trial. (?) indicates indefinite statement in paper reviewed.

membered that in many instances more than one reference relates to a single test. Both cold- and warm-blooded animals are listed as they were named in the literature, without regard to modern taxonomic placement. While many arthropod species have been tested for the presence of naturally acquired virus infections, deductions based on epidemiological factors have compelled workers to seek virus isolations principally from mosquitoes, ticks, and mites.

In these tables, demonstration of the presence or absence of virus during a particular trial is denoted by a plus or minus symbol with the appropriate bibliographic number. These symbols are qualitative and imply only that one or more tests were negative or positive as the case may be. The few parenthetic question marks are those placed by this author and indicate indefinite statements in the paper reviewed. Comparisons may be made with these tabulations and those given previously by other authors, of which the following are particularly informative: Davis (*87*), Cockburn et al. (*77*), Hammon et al. (*152*), Manzelli (*235*), and Syverton and Berry (*392*). The summaries of Manzelli are especially useful (*235*).

Table 1 indicates that mosquitoes have been used mostly in attempting to isolate viruses, of which the most numerous tests have been with WEE.

Table 2 lists certain arthropods (*A. aegypti*, *Aedes cantator*, *Aedes sollicitans*, *Aedes taeniorhynchus*, *Aedes vexans*, *C. tarsalis*, *Dermacentor andersoni*, and *Dermacentor variabilis*) which demonstrate rather uniform ability to transmit viruses directly by biting. In

Table 2. Results of attempted direct[1] virus transmission by bite of arthropod

Arthropods studied	Western equine encephalomyelitis	St. Louis encephalitis	Eastern equine encephalomyelitis
Aedes aegypti	+114, 172, 193, 198(?), 246, 398.	+45, 354; −18, 146	+87, 114, 345, 399
Aedes albopictus	+355		
Aedes atropalpus		+261	+87, 99
Aedes cantator	+247(?)		+87, 99, 247, 399
Aedes cinereus			−87
Aedes dorsalis	+233, 234; −152, 172, 212		
Aedes togoi		+260(?)	
Aedes nigromaculis	+233		
Aedes sollicitans			+87, 99, 247
Aedes taeniorhynchus	+195, 197		+99; −195, 197, 399
Aedes triseriatus			+87, 99
Aedes varipalpus	−305		
Aedes vexans	+193(?), 194		+87, 99, 399
Anopheles freeborni	−137, 152, 172		
Anopheles punctipennis		−146	−87
Anopheles quadrimaculatus	−247	−18, 423	−87, 399
Armigeres obturbans[2]		+261	
Culex apicalis			−87
Culex erraticus		−146	
Culex pipiens		+137; −18, 105	−87, 399
Culex pipiens pallens		+260, 261	
Culex quinquefasciatus			−24, 25
Culex salinarius			−87
Culex tarsalis	+145	+146	
Culex territans (restuans)			−87
Culex tritaeniorhynchus		+261	
Culiseta inornata	+137		
Culiseta melanura			−87
Mansonia perturbans			−87
Psorophora ciliata	−145		
Psorophora confinnis	−145	−146	
Psorophora cyanescens	−152		
Uranotaenia sapphirina			−87
Wyeomyia smithii			−87
Dermanyssus gallinae		+369	+24
Bdellonyssus sylviarum	−23	−23	
Dermacentor andersoni	+389, 390, 393		
Dermacentor variabilis	−124	+47, 48, 49	
Diamanus montanus	−152		
Echidnophaga gallinacea	−163		
Ctenocephalides felis	−152		
Triatoma sanguisuga	+124		
Siphona irritans	−172		
Simulium vittatum	−211		
Stomoxys calcitrans	−152		
Tabanus punctifer	−172		

[1] Direct transmission by bite is inferred, i. e., the arthropod became infected by biting a test animal which had been infected by various means. [2] Probably *A. subalbatus.*

NOTE: + or − preceding bibliographic number indicates positive or negative results during a particular trial. (?) indicates indefinite statement in paper reviewed.

table 3 a remarkably high number of records appear indicative of positive transmission by various methods. It is obvious that the main attention has been upon tests involving mosquitoes in contrast with other arthropods. As indicated in table 4, little work has been published on the detection of neutralizing antibodies of EEE in animals. Additional data on the detection of neutralizing antibodies in the serums of a wide range of Colorado small mammals are detailed in the paper by Hutson et al.[10] in which only 6 out of the 300 tests were positive (5 positive WEE and 1 positive SLE tests). As shown in table 5, very few isolations of viruses have been reported from animals other than horses. Virus has been isolated from

[10] Hutson, G. A., Howitt, B. F., and Cockburn, T. A.: Encephalitis in Midwest. VII. Neutralizing antibodies in sera of small wild animals—Colorado, 1950. Proc. Soc. Exper. Biol. & Med. 78: 290–293 (1951).