GUIDELINES FOR ARBOVIRUS SURVEILLANCE PROGRAMS IN THE UNITED STATES

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TABLE OF CONTENTS

CHAPTER 1
INTRODUCTION

Purpose of The Guidelines

Approaches to arbovirus surveillance in the United States vary from state to state (see Appendix I), and surveillance data are rarely comparable. Standardized data collected in a standardized fashion can document regional patterns in the spatial and temporal dynamics of disease activity. That information can be used to predict and help prevent major epidemics.

Our purpose is to provide guidelines for standardization of surveillance for mosquito-borne viral encephalitis. We emphasize predictive, proactive, and efficient methods whenever possible. Following a general discussion of the philosophy of surveillance and the range of available surveillance tools we present, in Chapter 2, recommended surveillance methods for each of the common encephalitides found in the U.S. In Chapters 3-6, we provide brief reviews of the biology and behavior of the vectors and vertebrate hosts of the major encephalitides. In the reviews we discuss only those biological and behavioral characteristics that are important to the surveillance effort. We also have tried to identify important research questions and areas where data are lacking. Finally, several appendices provide supplementary information on case definitions, techniques and equipment for mosquito surveys, and vertebrate surveillance methods. Rather than giving highly specific directions for each method, we refer readers to the original references for details. In addition, many state mosquito control associations or health departments publish guidelines for surveillance and control of mosquito-borne disease.[8,182,204]

General Considerations

Surveillance is the organized monitoring of levels of virus activity, vector populations, infections in vertebrate hosts, human cases, weather, and other factors to detect or predict changes in the transmission dynamics of arboviruses. A sound surveillance program requires a thorough understanding of the biology, ecology and interactions of the vertebrate and mosquito hosts. The transmission of arboviruses depends on these interactions. The data needed to estimate the risk of transmission to humans are rarely available within a single agency. **It is extremely important that the various data-collecting agencies actively communicate and exchange information**.

The impact of prevention or control measures on the course of a potential epidemic is diminished by even the smallest delays. Biologic and ecologic factors influence the temporal pattern and intensity of arbovirus cycles. Optimal environmental conditions allow rapid increase of vectors and virus amplification in vertebrate hosts. It is urgent, therefore, that a well-organized surveillance program be in place well in advance of the virus transmission season. Virus isolation and identification techniques are rapid and new sampling methods can quickly define the vector situation. Still, these procedures require considerable time and effort.

Enzootic virus transmission may occur only at a low intensity among certain vertebrate host and mosquito species within specific habitats in rural or suburban environments. Thus, transmission may remain undetected by most monitoring programs. However, when low host immunity and an abundance of vertebrate hosts and mosquitoes are synchronized with favorable weather conditions, transmission may increase in intensity and expand in distribution, producing an epizootic. If epizootics begin early in the transmission season and if epizootic foci expand into urban centers that possess adequate host and vector populations, the risk of human involvement increases.[178]

The prevention and control of arbovirus diseases depend upon identifying and monitoring vertebrate host and vector species involved in spring amplification and on monitoring the sequence of events and forces that lead to epizootics or epidemics. Enzootic vertebrate hosts and vectors also may be involved in epizootic or epidemic transmission. In Memphis, Tennessee, for example, many of the bird species that were involved in enzootic maintenance also participated in epizootic amplification of St. Louis encephalitis (SLE) virus[a].

A proactive surveillance system designed to provide early warning of epidemic activity should collect data on several variables rather than relying

[a] McLean, R.G. Unpublished data.

on a single predictor. Control measures should be started when a particular predictor exceeds the action threshold (usually determined from historical data and experience). For example, if early season climatologic data are compatible with epidemic activity, state and local agencies should make contingency plans. Such plans include contracting in advance for aerial ultra-low volume (ULV) insecticide application later in the season when, or if, needed. Ideally, the planning process involves other agencies and interest groups at the earliest possible time. This is the time to begin early-season control activities such as mapping larval habitats, source reduction and educating the public. Some or all of the following factors can increase the predictive ability of arbovirus surveillance programs: season, landscape ecology, meteorologic data, vertebrate hosts, vectors, and human case data.

Seasonal Dynamics

The *power* of a predictor is the likelihood that, if an outbreak is predicted, it will actually occur. There is a negative relationship between predictive power or accuracy and lead time between predictor and event. Predictions normally become more accurate as the season progresses, but provide less reaction time to carry out control measures to prevent human cases. By the time human cases are confirmed (a very accurate predictor), the epidemic may be waning of its own accord and control measures may have little impact.

Different measures or predictors for epidemic transmission are effective at different times of the year.[95,295] The earliest useful predictors are climatologic factors that influence size of the early mosquito population. These include fall, winter, and spring temperatures, rainfall, snowpack, runoff, and flooding, depending on the virus(es), vector(s), and region of the country.

Mid-season predictors usually consist of population estimates of vectors, and vertebrate hosts (especially young of the year), and evidence of early virus transmission in the natural cycle. The likelihood of an outbreak is estimated by comparing current vector and vertebrate host population densities and age structures with long-term averages. Late-season predictors consist of evidence of virus spill-over to sentinel bird/chicken flocks, epidemic/epizootic vectors, and domestic animals. The likelihood of transmission to humans or domestic animals becomes more accurate as virus begins to circulate in vector and vertebrate host populations.

Patch Dynamics and Landscape Ecology

Localities vary in geography, weather, plant cover, soil type, host and vector distribution, host immune status, etc. Likewise, conditions at a given locality change with time. This spatial and temporal variation (called patch dynamics[227]) makes it difficult to use a single criterion as a predictive measure over wide geographic areas[224] or even in one area over several years. Therefore, agencies will need to collect data in a range of different habitats over long periods (5 or more years) to improve the predictive capability of surveillance systems. Once long-term baseline data are available, it is more informative to express vector or host abundance indices as deviations ($\pm$ S.D. or S.E.) from the seasonally-adjusted (monthly, weekly) long-term mean index (e.g., as is done for stock market performance or volatility).

Meteorologic Data Monitoring

The great variety of local ecologic factors that influence transmission complicates the use of meteorologic data to predict epidemic arbovirus activity. Different vertebrate hosts and mosquito vector species respond to meteorologic changes in different ways, depending on geographic location and other factors.

In correlating meteorologic data with human disease incidence, problems arise from the focality of weather patterns, and the availability and appropriate choice of local weather data. For example, in correlating temperature and rainfall patterns with a statewide outbreak, which combination of weather stations does one choose as the data source? That is, at what *scale* should we examine the system? A second concern is the wide variations of temperature, precipitation and other indices that occur on a daily, monthly or annual basis. For a given station, the range in these observations may be extreme and the confidence intervals on the mean extremely broad. Deviations from the norm must, therefore, also be extreme to lie outside the normal limits. Combinations of less extreme deviations may be effective predictors. By comparing current measurements with long-term (e.g., 20-year averages) data, it is much easier to detect significant changes in these factors.

Certain wind patterns can carry agriculturally important insects to new, distant

locations.[139,181,261] Recently, interest has focused on the possibility that infected vectors species also are distributed in this manner. Trajectory analysis was used to match the geographic location of equine and human encephalitis cases with the convergence of southerly-moving warm fronts and northward-moving cold fronts.[256,257] Without large-scale mark-release-recapture studies, however, it is impossible to separate hypotheses based on wind-borne dispersal from hypotheses based on Hopkins' bioclimatic law. The bioclimatic law predicts seasonal retardation of biologic activity with increasing latitude and altitude.[134]

Vertebrate Host Surveillance

Wild vertebrates are hosts for at least 63 registered arboviruses in North America and hundreds more throughout the world.[3] Moreover, new viruses are discovered continually. In the U.S., however, only four mosquito-borne arboviruses--St. Louis encephalitis (SLE), eastern equine encephalomyelitis (EEE), western equine encephalomyelitis (WEE), and La Crosse encephalitis (LAC)--have had a significant impact on human health.

There are local and regional differences in vector and vertebrate host species, arbovirus strains, climate, habitats and urban development within the United States. Therefore, no single sentinel host species or specific surveillance technique is effective in all areas. For example, in west Texas, the number of WEE cases in humans was more highly correlated with virus isolation rates from house sparrows than with vector population densities or environmental conditions.[120,133] In California, the statewide surveillance program does not sample wild birds. Studies in that state found WEE virus isolations from *Cx. tarsalis*, seroconversions in sentinel chickens, and the incidence of WEE in humans all were positively associated with *Cx. tarsalis* abundance in light traps as indices rose to moderate levels. However, the relation became negative as light trap indices continued to rise.[224,237] Virus isolations from *Cx. tarsalis* generally preceded seroconversion in chickens.[237] Each local health agency should conduct initial surveys to get information on the relative abundance, potential reproductive activity, and infection rates in vertebrate host species.[125,179,234] This background information is used to design a surveillance system to fit local capabilities and needs.

Some general guidelines can be useful when an arbovirus surveillance program is in the planning stage. A separate publication gives detailed techniques for collecting and handling vertebrates and processing specimens for arbovirus studies.[279] That publication includes information on permits required for trapping wild animals. The characteristics that define good vertebrate hosts for arbovirus surveillance include the following:

1. Susceptibility to the monitored virus at rates that reflect virus activity in the surveillance area,
2. High titer and long duration of antibody response,
3. Low morbidity and mortality (except in those species where high mortality is easy to detect),
4. Locally abundant population,
5. Locally mobile to increase exposure to and dissemination of virus,
6. Frequent exposure to vector species (could overcome lack of mobility),
7. Attractive to and tolerant of vector feeding,
8. Easily captured by conventional methods,
9. Ease in handling and obtaining blood specimens,
10. Age determination possible, at least young of year, or the regular multiple captures of tagged animals permits detection of seroconversions,
11. Relatively long-lived for multiple sampling of same animal.

Probably no vertebrate species is universally suitable for arbovirus surveillance programs. Local abundance, distribution, exposure to vector mosquitoes, virulence of virus strains, and the competence of local vector species may vary regionally. For example, the house sparrow is a good sentinel for SLE virus in midwestern urban settings[165,178] and for WEE and SLE viruses in rural west Texas.[120,133] It is inadequate as a sentinel for SLE in Florida and California,[176,180] for WEE in rural areas in the northern plains states[179] or for EEE in southwestern Michigan.[177] Other species (e.g., the house finch in California[234]) can be used in those

areas. Conduct an initial survey to determine the most abundant local bird species exposed to the virus, the species that are easiest to sample, and the best sampling locations.[125,180,179]

Arbovirus surveillance programs throughout the United States use a variety of species of birds and mammals. Many other species have been sampled only once as part of a survey to discover which arboviruses were present or which species were tangentially infected. Exposure is increased in long-lived species (wild ungulates) or in those with high mobility or particular feeding habits (carnivores). These latter species may be useful in determining the presence, distribution, and annual prevalence of a virus. Serosurveys of wild ungulates have provided valuable information in several states (see Appendix III for examples).

SLE and WEE virus infections in birds strongly correlate with reported human cases caused by these viruses in the same area.[120,165,241,288] Some programs regularly sample passerine birds (e.g., house sparrows) or chickens every year during the transmission season to detect annual and seasonal changes in arbovirus activity. To provide more complete coverage of the surveillance area,[133,178] passerine and other free-ranging wild birds can be monitored in areas not covered by sentinel chickens. Some surveillance programs use free-ranging birds exclusively, some use only house sparrows, and others use a variety of wild bird species. The scope of such avian monitoring programs depends on the specific purposes and level of responsibility of the health department. Arbovirus surveillance programs may cover only metropolitan centers, may be regional programs covering parts of states, or they may be statewide.

Captive sentinel animals are used to establish the presence of arboviruses and to monitor temporal and spatial changes in virus activity in an area. Sentinels are sometimes used to attract mosquitoes for virus isolation. The use of sentinel animals allows flexibility. The primary advantage of using captive sentinels is that the time and place of exposure are known. The use of sentinels also assures uniformity in selection of location, habitat, number, breed, age and source of the animals, and sampling schedule. Seroconversion and field infection rates are reliably determined when the foregoing factors are controlled. The disadvantages of sentinel animals include the expense of buying animals, building shelters or cages and maintaining the animals in the field. Also, the lack of mobility of sentinel animals affects their exposure to mosquitoes, and limits the geographic area represented. The following paragraphs discuss the common species used as sentinels.

Domestic chickens: Probably the most widely used sentinel animal for WEE and SLE surveillance is the domestic chicken. Chickens are attractive hosts for *Culex* mosquito vectors. They are susceptible to and can tolerate arbovirus infections, and they produce readily identifiable antibodies. Older birds are unlikely to contribute to local virus amplification because they usually develop only low titered viremia. Chickens are hardy and are easily handled and bled. They are inexpensively maintained on farms or in urban-suburban locations by residents or health officials. Eggs laid by the birds may provide an added incentive and help to defray any costs of maintaining the birds.

Six- to eight-week-old chickens are obtained in the spring. Each monitoring site is stocked with 10-30 pretested, non-immune, individually-banded birds. Dispersing smaller groups of birds throughout the area at risk yields a more representative estimate of arbovirus activity. It is important to base the choice of locations for sentinel chickens on historical records of virus activity, vector resting sites or flight corridors, and the likelihood of virus transmission rather than on convenience. The chickens are kept in standard sentinel sheds or similar structures.[231,279]

Sentinel chickens are bled from the wing vein, the jugular vein, or from the heart biweekly or monthly throughout the transmission season. Seroconversions may occur 2-3 weeks before the detection of equine or human cases of WEE and weeks before human cases of SLE. If the intent of surveillance is to monitor season-long transmission, birds that seroconvert to positive are replaced by non-immune birds, preferably of the same age. In areas of low intensity of virus activity or where the only objective is to detect initial transmission, replacement is unnecessary since most individuals are still susceptible. All birds are still useful if more than one arbovirus is present in the surveillance area.

Sentinel chickens are used extensively for arbovirus surveillance.[130,156] Currently, a few states like Delaware, Florida, California and Utah use sentinel chicken flocks scattered throughout the

areas of greatest risk for EEE, SLE, or WEE infection. Sentinel chickens were not useful for monitoring EEE virus activity in New Jersey.[63]

Free-ranging wild birds: Wild birds, principally passerine species, are the primary vertebrate hosts of SLE, EEE, and WEE viruses and serve as the principal hosts for mosquito infection. Virus activity and antibody seroprevalence for these viruses in local bird populations usually correlate well with the risk of human infection. Accurate monitoring of virus and antibody prevalence in wild birds should provide early warning of increased transmission that may constitute a risk to the equine and human populations.

Wild birds are monitored by repeated sampling of local populations to test for antibody or virus. Free-ranging adult and immature birds are captured in ground-level mist nets set at locations appropriate for the desired species. The Australian crow trap [181] also provides an effective method for collecting birds. Captured birds are bled, banded, and released for possible later recapture to check for seroconversions. Recapture data also gives useful insights on movement, survival, and other population characteristics of the birds. Successful use of this technique requires an intensive sampling effort because of low recapture rates. Since antibodies may persist for 2 or more years, the results from carefully identified juvenile birds may provide the most useful index of current virus activity.[269] This technique is costly. It requires highly trained personnel as well as state and federal collecting permits.

Detection of viremia in nestling birds during the summer transmission season has been successfully used in WEE and SLE surveillance.[120,125,133,179] Nestling birds are more susceptible to certain arboviruses than adults. They may produce viremia of longer duration and higher titer, providing a valuable early season indicator of transmission intensity.[132] Additional information on location, reproductive stage, cycling of broods, and local abundance can be obtained from a survey of nesting activity.[179,190]

House sparrow nestlings are a sensitive indicator of recent transmission, and are particularly useful in locations where they are the predominant avian species. They live in peridomestic settings, and are attractive to and frequently bitten by *Culex* mosquito vectors. The adults' gregarious behavior leads to nests being clustered at specific locations, so nestlings can be sampled easily. Virus isolations from house sparrow nestlings occurred early in the transmission season and correlated well with later human cases of WEE and SLE in Texas.[120,125,133] Nestling birds of other species such as pigeons, house finches, barn swallows, and mourning doves also may be valuable indicator hosts when abundant. These species could supplement or replace house sparrows as sentinels.

Equines: Surveillance for equine cases in areas with susceptible horse populations may provide the most practical and sensitive tool for the recognition of a potential public health problem caused by EEE and WEE viruses. This is especially true in areas that lack the resources to monitor virus activity in birds and mosquitoes. As a result of their field exposure, horses are subject to high vector attack rates. Equine surveillance can be active or passive. Reports by local veterinarians of equine encephalomyelitis give warning of increased arbovirus activity in an area.[37] This can alert public health officials to investigate the situation. Active surveillance requires regularly contacting large-animal veterinarians, encouraging them to report clinically suspect equine cases, and to submit blood and autopsy samples for laboratory confirmation. Record sheets, containing a case history and vaccination history, must acompany samples for laboratory testing if the results are to be useful. Some limitations in using equines are their vaccination status, movement into and out of the surveillance area, and lack of prompt reporting of morbidity by attending veterinarians.

Other domestic and wild mammals: Wild mammalian hosts are used as sentinels for California serogroup viruses. New Zealand white rabbits stationed in wire cages in wooded areas in eastern Canada confirmed local transmission of snowshoe hare (SSH) virus.[174] Domestic rabbits, eastern chipmunks, and red foxes have been used as sentinels in the north-central states to monitor LAC virus transmission.[109,305] Domestic rabbits[144] and cotton rats were used to detect transmission of Keystone (KEY) virus in the southeastern United States.[282] Cotton rats also were used in overwintering studies of SLE virus in the southeast and might be useful in a surveillance program.[176] State-wide surveillance for Everglades virus (EVE) activity in Florida used raccoons.[29]

Appendix III describes several local and

state surveillance systems that use vertebrates. It also lists species of birds and mammals that have been used in arbovirus surveillance programs throughout the U.S.

Mosquito Surveillance

Mosquito surveillance should have two basic activities, 1) identifying and mapping larval habitats and 2) monitoring adult activity.[35,48] Both activities provide useful information in a proactive arbovirus surveillance system. Mapping and monitoring larval habitats gives early estimates of future adult densities and, under some conditions, provides the information necessary to eliminate mosquitoes at the source. Monitoring species, density, age structure, and virus infection rates in adults provides critical early, predictive data for the surveillance system.

Adult sampling stations usually should be located well away from larval habitats to reduce the number of males and young (nulliparous) females. Alternatively, the program can use gravid traps if they attract the species of interest. A high proportion of males in a collection usually indicates a nearby larval habitat. Data from both larval and adult collections are plotted to show mosquito density as a function of time for each station. Use these data to schedule control efforts and to evaluate control efficacy. Population changes are clearer when abundance is plotted on a logarithmic scale.[25]

Well-prepared and maintained larval habitat maps to provide long-term baseline data. Maps are updated throughout the season to show the location of mosquito breeding sites and locations with high adult densities. Several automated data collection systems, using hand-held microcomputers, ease data collection and speed up the response to newly discovered larval habitats.[b] State and local agencies also can use computer-based geographic information systems (GIS) for a variety of planning and decision-making tasks.[7] City, county, and state planning commissions frequently operate GIS programs and have extensive databases. GIS systems can greatly speed and simplify the process of mapping larval habitats, location of known virus foci, urban centers at risk, planning emergency response activities, etc. When several users share the cost of obtaining the data, GIS can be a highly cost-effective means of mapping and planning.

Except when transovarial transmission is a major part of the enzootic cycle (as with LAC virus), the maintenance and transmission of arboviruses is strongly dependent upon adult female survival rates.[86,100] It is more likely that older females have fed, acquired virus, and lived long enough to become infective. Surveillance programs often assume that older females are present at some more-or-less constant proportion in the total population (i.e., a stable age-distribution) and, therefore, that the total trap count has a direct relation to arbovirus transmission activity.[185,224] Frequently this is not a valid assumption. For example, as larval populations increase, competition for resources also increases. The availability of nutrients in some larval habitats can vary during a single season, further compounding the effects of competition.[101,259] Adults that emerge from highly competitive situations are smaller and less robust. The reduced adult survival rate leads to proportionately fewer old adults in the population.[1,163] Adult longevity, therefore, is dependent on larval population density. Thus, there is likely to be a stronger correlation between abundance of old vectors and arbovirus transmission rates than between total vectors and transmission.[88,235]

Good estimates of changes in the density of parous females, not just of the total vector population, can improve the predictive capability of mosquito surveillance. In New Jersey's EEE surveillance program, percent parity in *Ae. sollicitans* is determined by ovarian dissections.[64] To selectively sample older components of the vector population, susrveillance programs should use female-retaining gravid traps (see Appendix II) instead of light traps whenever such traps are appropriate for the species being sampled.

Human Case Surveillance

The primary purpose of a surveillance system is to provide information to direct prevention and control activities. The surveillance system has no value if the data collected are not used to implement control measures in a timely fashion. Arbovirus surveillance requires input from many different agencies. Coordination and sharing of data between those agencies are essential for the surveillance system to function properly. State and local public health officials need to be contacted immediately if evidence is found of increased arbovirus activity in a

[b] Street, L.J. 1986. Larval data collection program for the HP-71B. Unpublished programs. Chatham Co. Mosquito Control Commission, Savannah, GA.

mosquito, avian, or equine population. Similarly, vector control officials should be contacted when a suspected human case of arboviral encephalitis occurs so additional environmental monitoring and appropriate control strategies can be planned.

At the national level, the Division of Vector-borne Infectious Diseases (DVBID), Centers for Disease Control and Prevention (CDC), collects information from the states on cases of arboviral encephalitis. Although state and federal laws do not require physicians or hospitals to report human cases, there has been good cooperation between local, state and federal agencies in reporting cases of arboviral encephalitis.

Standardized report forms and electronic reporting systems are used by state epidemiologists to notify CDC of most reportable illnesses. Forms with demographic, clinical, and epidemiologic information are used to determine whether patients meet the surveillance case definition. Case definitions for the common arboviral illnesses found in the United States are published periodically (see Appendix I).[52] Although the routine reporting of human cases of encephalitis was discontinued in 1983, many states still report cases and other relevant data, on an informal basis, using the forms shown in Appendix I. Since 1983, DVBID has informally collected information on human arbovirus cases by telephone from state and local agencies. This surveillance system is useful for immediately identifying possible outbreaks of arboviral disease. However, it is very time-consuming, and detailed epidemiologic data on cases of arboviral illness are seldom available. CDC is currently revising human surveillance procedures for arboviral encephalitides to include reporting cases electronically using a standardized report format based on the forms shown in Appendix I.

Arboviral illnesses are widely under-reported in the United States.[285] These illnesses have varied clinical presentations that cannot be clinically distinguished from other forms of viral encephalitis, and serologic testing is therefore critical for diagnosis. Because there is no specific therapy for these illnesses, local physicians are often reluctant to obtain samples for serologic tests. Moreover, they must be regularly reminded of the public health importance of arboviral disease outbreaks and encouraged to report suspected cases to state and local health departments rapidly so that investigations and control can be initiated if necessary.

Because several arboviral illnesses have a high inapparent-to-apparent infection ratio, the prevalence of arbovirus antibodies can be high in some populations. A diagnosis of arboviral encephalitis requires that the patient have signs and symptoms compatible with neuroinvasive disease. For reporting purposes, clinical data should be obtained to ensure that the patient meets the criteria for the surveillance case-definition (see Appendix I).[52] From patients with such signs and symptoms, physicians should obtain both acute phase (1-7 days post-onset) and convalescent phase (>14 days post-onset) serum and cerebrospinal fluid specimens.

When a case of suspected human arboviral encephalitis is reported, the individual's site of exposure and the risk of additional human cases should be assessed. The patient's age, sex, race, and place of residence should be recorded. To determine sites of possible exposure and risk factors for illness, data can be collected on:

a) recent travel to areas with known viral activity in mosquito populations,
b) peridomestic, neighborhood, occupational, or recreational exposure,
c) conditions that promote peridomestic mosquito breeding (e.g., empty tires and containers), and
d) conditions that increase contact with vectors (e.g., gardening, lack of air conditioning).

Even if the immediate danger for other human illnesses seems remote, these data should be sought to provide a basis for future control measures. This list is not meant to be exhaustive, and the epidemiologic data collected should be tailored to each arboviral illness under consideration.

When an outbreak is suspected or anticipated, increased surveillance for human cases should be considered. Special surveillance measures that might be initiated include undertaking active surveillance for encephalitis or meningoencephalitis admissions to local hospitals and enhancing the testing of undiagnosed encephalitis patients. Contacting local physicians and infection control nurses about the need for arbovirus testing and reporting of all suspected cases will increase the sensitivity of the surveillance system to detect cases of arboviral encephalitis. This can be accomplished through direct mailings, participating in local hospital meetings and grand rounds, and giving lectures/seminars to local medical groups. Special studies to detect unrecognized cases, such as routine

testing of all cerebrospinal fluid samples drawn during the transmission season, should also be considered. Private diagnostic laboratories also should be included in the list of contacts.

Increased or early arbovirus activity in animal populations may herald an upcoming outbreak of arboviral illness in humans. Five risk categories for arbovirus outbreaks have been defined and appropriate responses established (Table 1). Data collected in vector control investigations may be useful in determining a qualitative probability of an epidemic as well as a stepwise response to this threat. In addition, knowing the type of infected vector, the predominant type of arbovirus, and the location of viral activity may help state and local health departments provide a more focused public health message to groups at high risk for infection. It is critical, therefore, that vector control/surveillance specialists work closely with health department officials to ensure that data can be analyzed and used to direct an appropriate response as early as possible.

Locally relevant predictors of arboviral disease in humans may be obtained if human surveillance data can be correlated with sentinel surveillance data.[224] Parameters of arbovirus activity in defined geographic areas, such as census tracts or mosquito abatement districts, may be collected routinely and consistently over a period of several years by vector control personnel. These data then can be correlated with human arbovirus infections occurring within the same areas during the same time period. With this information, sensitivity, specificity, and positive predictive value calculations can be made to predict subsequent cases of human disease. Such models may be useful in predicting the eventual occurrence of a human outbreak and instituting control measures prior to the appearance of human illness.

Evidence of increased or early arbovirus activity in animal populations may herald an outbreak of arboviral illness in humans. Data collected in vector control investigations can be useful to health departments that monitor human populations for the occurrence of cases. Knowing the vector species, the virus, and the location of viral activity should help health departments to provide a more focused public health message to groups at high risk for infection.

Natural disasters and encephalitis outbreaks: Natural disasters such as floods and hurricanes can create a potential for epidemics of vector-borne disease. When a response to these disasters or emergencies is beyond the capability of state or local governments, the president may determine that a disaster or emergency exists. A presidential disaster declaration makes state and local agencies eligible for reimbursement of disaster-related expenses. The Federal Emergency Management Agency (FEMA), which oversees all federal disaster activities, calls upon CDC to evaluate the risk of vector-borne disease. Reimbursement for vector control depends on the presence of a clear risk of vector-borne disease that can be related to the emergency or disaster.

In order for CDC to rapidly and accurately evaluate the risk of vector-borne disease, it is important for state and local health and vector control agencies to have readily accessible as much data as possible. Historical data should be available for comparison with current data, to show how the disaster is related to any increase in vector or virus activity. The types of information that are needed to estimate the risk of an epidemic are the following:

a) ***Mosquito population indices*** (Are vector species present? How do light trap indices compare with previous years and with this year prior to the current disaster?)

b) ***Virus infection rates in mosquitoes*** (What is the minimum infection rate (MIR) this year? How does it compare with MIRs in epidemic years? Is virus activity localized or is it widespread?)

c) ***Evidence of increased virus transmission in vertebrate amplifying hosts*** (What temporal and spatial patterns are seen and how do they compare with the norm for this locality?)

d) ***Evidence of disease in equines*** (WEE/EEE)

e) ***Rainfall and temperature data*** (Is there any evidence to show an association between past outbreaks/epidemics and specific weather patterns?)

f) ***Time of year*** (Is it relatively early in the virus transmission season for this locality?)

g) ***Risk to the human population*** (Is virus

activity near populated areas? Is vector movement between areas of virus activity and populated areas?)

If all of the foregoing information is readily available, a rapid risk assessment can be made using the categories in Table 1. If insufficient information is available, it is necessary to collect at least part of the data before a decision can be made. This frequently delays efforts by state or local agencies to implement the appropriate response. The delay may, in turn, result in increased virus and vector activity and human or equine encephalitis cases. State and local agencies should consider the components of Table 1 and points a) through g) above in designing surveillance programs.

Table 1.1. Definitions and stepwise response for risk categories for mosquito-borne arboviral disease outbreaks in the United States. Risk categories are tentative and approximate. Local and regional characteristics may alter the risk level at which specific actions must be taken.

Category	Probability of outbreak	Definition	Recommended response
0	Negligible or none	Off-season; adult vectors inactive; climate unsuitable	None required; may pursue source reduction and public education activities
1	Remote	Spring, summer, or fall; adult vectors active but not abundant; ambient temperature not satisfactory for viral development in vectors	Source reduction; use larvicides at specific sources identified by entomologic survey; maintain vector and virus surveillance
2	Possible	Focal abundance of adult vectors; temperature adequate for extrinsic incubation; seroconversion in sentinel hosts	Response from category 1 plus: Increase larvicide use in/near urban areas; initiate selective adulticide use; increase vector and virus surveillance
3	Probable	Abundant adult vectors in most areas; multiple virus isolations from enzootic hosts or a confirmed human or equine case; optimal conditions for extrinsic incubation and vector survival; these phenomena occur early in the "normal" season for viral activity	**Implement emergency control contingency plan:** Response in category 2 plus: Adulticiding in high risk areas; expand public information program (use of repellents, personal protection, avoidance of high vector contact areas); initiate hospital surveillance for human cases
4	Outbreak in progress	Multiple confirmed cases in humans	**Continue with emergency control contingency plan:** Concentrate available resources on strong adulticiding efforts over areas at risk; hold daily public information briefings on status of epidemic; continue emphasis on personal protection measures; maintain surveillance of vector/virus activity, human cases

In addition to federal disaster assistance provided through FEMA, some states have established their own funding procedures for vector-borne disease emergencies. Similar requirements for supporting data may be required for access to state emergency funding.

Laboratory Methods to Support Surveillance by Local and State Health Units

The choice of laboratory diagnostic tests depends on the needs, approach, and surveillance philosophy of a given health agency. The most commonly used methods include direct and indirect fluorescent antibody (DFA and IFA) tests, hemagglutination-inhibition (HI), complement-fixation (CF), neutralization (N), and IgM and IgG enzyme-linked immunosorbent assay (ELISA) for detection of antibody.[38,39,40,41] Antigen-capture ELISA[94] is used for direct detection of antigen in mosquito pools, and in human and animal tissues. Various cell cultures[42] or baby mice are used for virus isolation. The most common methods used to identify virus isolates are DFA, IFA, CF, N, or ELISA. Although it is not yet available for routineuse, the polymerase chain reaction (PCR) shows promise as a rapid and specific arbovirus detection method.[157]

Specimen collection: Specimens may consist of whole blood, serum, cerebrospinal fluid, or tissue samples. These should be processed immediately or placed on dry ice (-70°C) or other suitable deep-freezing agent if virus isolation is to be attempted. Although this may not be critical for antigen detection, shipment and storage of specimens at low temperatures prevents further degradation of proteins. Serum specimens to be tested only for antibody can be shipped at ambient temperatures for brief periods, provided they are collected aseptically and kept free of contaminating microorganisms. If transit time to the laboratory is longer than several days, refrigeration or the addition of antibiotics is necessary to prevent deterioration of the specimen.

Human serum: One or more of many methods are used for detecting antibody in human serum (see above). Laboratory confirmation of clinical diagnosis depends on direct detection of antigen, virus isolation, or serologic tests. However, the likelihood of SLE, EEE, WEE, LAC, or other arboviral encephalitides being isolated from blood or spinal fluid taken during the acute stage of illness is usually not great. Often the viremic stage has passed before the individual becomes ill. This is not the case with a few viruses for which humans are the principal viremic host in the transmission cycle (dengue fever and yellow fever). These latter viruses may be consistently isolated during the first 5 or 6 days after onset of symptoms.[113] SLE virus may be isolated more often from, or antigen detected by immunofluorescence in, brain collected post-mortem.

Antibody generally is not detectable until the end of the viremic phase. Detectable IgM antibodies usually appear soon after onset of illness and usually persist for only a few months. Their presence can serve as an indicator of recent infection. Detectable IgG antibody appears shortly after IgM and contains antibodies by neutralization, HI, and CF. IgG antibody produced after infections with arboviruses persists for months, years, or even for the life of the individual. Therefore, the presence of IgG antibody does not necessarily denote an active or recent arbovirus infection. The fetus or neonate produces IgM, but not IgG in response to infection *in utero* or shortly after birth. The large size of the IgM molecule prevents it from crossing the placenta. Thus, the presence of IgG in the fetus or neonate indicates passive transfer of IgG across the placenta.

Measurement of IgM antibody in cerebrospinal fluid is extremely useful for serodiagnosis. Because IgM antibodies do not cross the blood-brain barrier, finding IgM antibodies in cerebrospinal fluid implies intrathecal antibody synthesis in response to central nervous system infection. Moreover, the titer of IgM antibody in cerebrospinal fluid may be a prognostic indicator in certain encephalitides. However, IgM antibodies to some viruses have been detected for long periods, and a minority of patients may have prolonged IgM antibody responses. This limits somewhat the value of these assays as a measure of very recent infection. IgM antibodies seem relatively type-specific for arboviral encephalitides, but complex- and serogroup-reactivity also are observed.

HI antibody is broadly reactive among viruses of a serogroup, making this a useful test for preliminary screening. CF antibody is more complex-specific, short-lived, later to appear, and of lower titer than HI antibody. Finding antibody to a particular virus by CF usually indicates the individual was recently infected with that or a

closely-related virus. Certain individuals infected with arboviruses never produce CF antibody, or produce it too late to be of diagnostic value. Nevertheless, the presence of CF antibody in a patient can be used as presumptive evidence of recent infection. As with HI and NT tests, a fourfold rise in titer between paired acute- and convalescent-phase serum samples is confirmatory of infection with that or a closely related virus. CF tests now are considered relatively insensitive for antibody detection and, unfortunately, are no longer widely used. Because birds do not produce CF antibodies, the CF test is not useful for determining antibody in this group of animals.

The HI, CF, and IgM antibody capture (MAC) ELISA tests are not virus-specific. The MAC ELISA is at present, and for the foreseeable future, the test of choice for making provisional serodiagnoses with single serum specimens or with cerebrospinal fluid. It is of great value even when paired acute- and convalescent-phase serum samples are available. The MAC ELISA is comparatively easy to perform, and can be used to test large numbers of serum samples. Furthermore, the presence of IgM antibody usually signifies recent infection, the *sine qua non* of surveillance.

Bird and wild mammal sera: Specimens usually are tested for antibody to detect changes in population immunity. This provides evidence for virus amplification in a population. As with human serum, antibody is determined by one or more of the following tests: IFA, HI, IgM and IgG ELISA, and N. N tests are the most sensitive and specific, but are costly and complex to perform. IFA, HI, and IgM ELISA tests often are used to screen serum, with N tests used for confirmation of positive and negative specimens.

Virus identification: No single virus isolation system is adequate for all arboviruses. More sensitive isolation systems (inoculation of mosquitoes *in vivo*, inoculation of arthropod cells *in vitro*) are being increasingly employed.[250] It is becoming apparent that there are many virus strains or viruses that have not been detected because of the bias incurred by use of traditional systems, such as suckling mice and vertebrate cell cultures.

Traditional methods for virus isolation are still used in many laboratories. Suckling mice have been used as laboratory hosts for amplifying virus in diagnostic specimens and from field-collected mosquitoes, ticks, and animal tissues. They are inoculated intracranially with clarified suspensions of specimens. Because suckling mice are available to nearly all laboratories, particularly those that isolate rabies virus, this system holds certain advantages over others. Nevertheless, mosquito cell cultures, particularly C6/36 (*Aedes albopictus*), AP-61 (*Aedes pseudoscutellaris*), TR-284 (*Toxorhynchites amboinensis*), and other cell lines are increasingly being used for virus isolation.[111,155]

Arthropod cell culture systems have the advantage of ease of containment and reduction of aerosols. These cell lines are highly stable and have optimal growth at lower temperatures than do mammalian cells. Cultures and mosquitoes may be taken to the field, inoculated with clinical specimens, and returned to the laboratory days or even weeks later, during which time virus amplification has occurred. For several viruses, mosquito cell cultures are more sensitive than mice or mammalian cell culture systems for virus isolation. However, they have the disadvantage in some cases of not producing cytopathic effects. Thus, they require secondary steps such as IFA to detect the presence of virus in the culture. Intrathoracic inoculation of *Toxorhynchites* and male *Aedes* mosquitoes, which do not take blood meals but in which dengue and other viruses replicate, have also been used with sensitivity and safety.[112]

The classical procedure for the initial isolation and identification of an arbovirus begins with inoculation of suckling mice or a cell culture system in which cytopathic effects or plaques develop. The isolate is characterized by testing its ability to pass through a filter that excludes bacteria and its sensitivity to lipid solvents such as ether, chloroform, or sodium deoxycholate. It is often useful to determine the pathogenicity of the agent for, and titers in, various laboratory animals and cell cultures. A crude alkaline extract or partially purified (sucrose-acetone extracted) antigen is prepared for use in serologic tests. The antigen is tested for its ability to agglutinate the erythrocytes of male domestic geese (*Anser cinereus*) and to react in CF tests with homologous antibody preparations. The antigen is then tested by HI or CF with a battery of antibody preparations. The test will include antibodies to: a) viruses representing various serogroups, b) viruses suspected as the etiologic agent of the disease, and c) viruses known to be present in the area in which the specimen was collected or in which the patient

contracted the illness.

The best method for identifying an arbovirus is one that is rapid, specific, and inexpensive. In some laboratories, electron microscopy can be used at an early step to provide an identification at the family level. This can greatly facilitate later characterization. The application of DFA or IFA tests using polyclonal or monoclonal antibodies can provide a rapid and simple means of virus identification. Because a complete battery of reagents is not yet available, this method is only used for the identification of certain viruses at present. Both DFA and IFA tests have been applied to direct detection of viral antigen in clinical specimens.

Once the isolate is characterized to the level of serogroup or antigenic complex by these less specific assays, N tests are performed with antisera against individual viruses to confirm the identification. If necessary, an antiserum is also prepared against the isolate and cross-tested against antigens of viruses in the serogroup to which it belongs. Most of the data regarding antigenic characterization of arboviruses have been generated using these tests. They remain the standards by which newly isolated viruses are to be judged. Newly developed reagents and procedures will add significantly to our diagnostic armamentarium and expand our ability to more fully characterize the epitopes and other antigenic moieties of viruses. For example, monoclonal antibodies are available with group-specificity against many arboviruses. In addition, antibodies have been characterized that show complex-reactive as well as type-specific and even strain-specific reactivities.

Virus is amplified in an *in vitro* system (C6/36, Vero, other cells), in baby mice inoculated intracranially or in mosquitoes inoculated intrathoracically. The virus is detected by DFA, IFA, antigen-capture ELISA, CF, or N tests. If facilities are available in the local or state health laboratory, definitive identification can be done with reagents obtained from CDC. Alternatively, unidentified or provisionally identified viruses can be submitted to CDC for further studies. Tests performed at CDC include those for biologic characterization (host susceptibility, titer, presence of hemagglutinin, presence of essential lipids, etc.) and IFA, CF, and N tests for definitive taxonomic placement.

Although this general approach has been used successfully for decades, various adaptations of the ELISA test are being applied to virus (antigen) detection and identification. Direct detection of viral nucleic acid using molecular probes (polymerase chain reaction, hybridization) is now being used to detect viruses directly. Furthermore, gene sequencing is used for molecular epidemiologic studies of viruses. Nevertheless, N tests are recommended for definitively identifying viruses that have been provisionally identified by HI, CF, IFA, and ELISA or detected directly.

CHAPTER 2
SURVEILLANCE RECOMMENDATIONS

General Considerations

Surveillance systems quantify disease activity at a given time, predict the probable future course of the disease cycle, and indicate when control should be started to prevent epizootic or epidemic transmission. This requires that surveillance programs be long-term, proactive projects, gathering and analyzing data in epidemic and nonepidemic years to provide a basis for setting thresholds and decision making. No single technique can collect all of the data needed for a rational assessment of the risk of vector-borne disease.

Because arbovirus cycles are complex, and components of the cycle vary regionally, threshold levels and indicator parameters must be determined individually for each surveillance region. Current-year data should be compared with historical data for the same region or locality, rather than looking for absolute index values. The appearance of human or equine cases is unlikely to be associated with a specific value of a single index (e.g., vector females per light trap night) over large geographic areas. However, such indices may prove locally useful.

The following is a brief summary, by disease, indicating the methods we feel are most appropriate for an ideal surveillance program. The realities of local, state, and regional resources will often restrict the extent to which these recommendations can be fully implemented. For an overview of the types of surveillance systems currently employed in various states, see Appendix I.

Eastern equine encephalitis (EEE)

The distribution of EEE is intimately associated with the distribution of the enzootic vector, *Cs. melanura*. Thus, the presence of this mosquito, or of habitat capable of supporting this species marks areas with the potential for EEE transmission. The density of *Cs. melanura* has often been related to the intensity of EEE activity. However, monitoring *Cs. melanura* population density alone is not a reliable surveillance tool; other mosquito species are responsible for transmission to horses and humans. In addition, a susceptible bird population is required for amplification of the virus. Successful EEE surveillance programs will monitor components of both the enzootic cycle (vector population, bird population, virus prevalence) and of the epizootic cycle (bridge vector populations).

Meteorologic data: Both local and regional weather patterns are important. The ideal program will monitor rainfall and temperature patterns that promote the development and survival of large mosquito populations, especially *Cs. melanura*, in each area. It should examine annual rainfall patterns for the previous 2-3 years. It should compare monthly rainfall quantities to local and regional averages, especially during fall and spring. It also should look for early temperatures that permit mosquito development. At least in the northeast, programs will monitor ground water levels in freshwater swamps as a method of predicting subsequent *Cs. melanura* populations.

Vector data: Surveillance programs should monitor current and historical patterns in density and age structure of *Cs. melanura* populations in swamp foci. Collections of *Cs. melanura* are made by using CO_2-baited CDC light traps and black resting boxes are effective for collecting *Cs. melanura*. Parity rates can be determined with sufficient accuracy to establish crude age structure by using the tracheation method of Detinova.[80] The program also should monitor field infection rates in *Cs. melanura* populations by submitting pools to the state or regional laboratory for virus isolation.

The ideal surveillance program also will monitor the density and age structure of epizootic vector species. These include *Cq. perturbans* and *Ae. canadensis* in swamp habitats, *Ae. vexans* in upland floodwater sites near swamps, and *Ae. sollicitans* in areas where enzootic foci are adjacent to coastal salt marshes.

Vertebrate host data: The ideal surveillance program will measure the prevalence of EEE viral antibody in wild passerine birds located near swamp foci during the current season (monthly) and compare to EEE antibody levels during the previous 2-3 years.

Other data: In areas where they are known to be effective predictors, seroconversion in sentinel chickens should be monitored. Programs should conduct active or passive surveillance for EEE in unvaccinated horses.

La Crosse encephalitis (LAC)

The LaCrosse virus cycle differs somewhat from that of other viruses discussed here. The primary vector is the tree hole mosquito, *Ae. triseriatus*. The virus is maintained in a focus by vertical (transovarial) transmission in the mosquito. The primary amplification hosts are chipmunks and squirrels. The virus is limited to wooded areas by the ecological requirements of the mosquito and vertebrate hosts. *Ae. triseriatus* does not disperse great distances from wooded areas. Human cases of LAC have been associated with the presence of artificial containers (i.e., discarded tires) in adjacent wooded areas. These containers can produce very large *Ae. triseriatus* populations.

Meteorologic data: The relationship, if any, between rainfall and *Ae. triseriatus* density is not known, but frequent rainfall will repeatedly flood treeholes and containers and produce frequent hatches. Therefore, surveillance programs should monitor seasonal rainfall.

Vector data: The density and field infection rate of *Ae. triseriatus* should be monitored. Adults can be collected at bait or resting in the understory of the woodlot. Ovitraps can be used to determine the number of eggs produced by the population. Eggs from the ovitraps can then be used to determine the proportion of offspring transovarially infected with LAC. Because ovitraps compete with naturally occurring oviposition sites for egg deposition, results should be interpreted with caution. Ovitrap results are useful for comparing density within a site over time, but comparisons of population density between woodlots are not reliable. Discarded tires and other artificial containers often serve as LAC virus foci near human habitations, and these should be inspected. Where *Ae. albopictus* is abundant, collect and process specimens for virus isolation.

Vertebrate host data: The ideal surveillance program will monitor current and historical patterns in presence, density and seroconversion rate of chipmunks and tree squirrels in LAC virus foci.

Other data: Surveillance data can be supplemented by serosurveys of humans living near LAC foci. Areas at greatest risk can be identified and mapped by identifying hardwood forest habitats where *Ae. triseriatus* and chipmunks or squirrels are abundant.

St. Louis encephalitis (SLE)

At least three, and probably four, geographically distinct patterns of SLE transmission can be distinguished, based on the primary vector species (see Chapter 5). Techniques used to monitor SLE activity will vary depending on whether the vector is *Cx. tarsalis, Cx. p. pipiens, Cx. p. quinquefasciatus, or Cx. nigripalpus*.

Meteorologic data: The amount of rainfall, interval between rainfall events (Florida), and January - July cumulative precipitation (California) have been useful predictors of SLE activity. Complex seasonal temperature and rainfall patterns have been found for SLE transmitted by *Cx. pipiens* complex mosquitoes.[247]

Vector data: Surveillance programs should sample populations of the important local vector or vectors (Appendix II lists sampling methods for particular species). Mosquito pools should be submitted for arbovirus isolation to a state or regional laboratory. Programs should monitor vector abundance in peridomestic container habitats when *Cx. pipiens* complex is involved in transmission.

Vertebrate host data: Passeriform and columbiform birds that are locally important in the enzootic SLE cycle (see p. ?) should be bled to obtain serum samples. Programs may or may not choose to use sentinel chicken flocks, depending on whether seroconversions precede or are concurrent with human infections. This appears to vary with region and vector species.

Other data: Using census maps, the program should identify areas characterized by large elderly populations or by low socioeconomic status, as clinical disease tends to be more frequent in these locations.

Western equine encephalitis (WEE)

Cx. tarsalis is the primary vector of WEE throughout the range of the virus. Thus, the ecology of WEE is more uniform than with arboviruses that have regionally differing vectors. Differences in disease dynamics are more likely to be linked to north-south seasonal differences in temperature and rainfall. Differing enzootic avian hosts also may alter the dynamics of WEE transmission.

Meteorologic data: The ideal surveillance program will monitor meteorologic data to estimate the likelihood of increased WEE activity. In California, climatologic data provide an early-season gauge of the likelihood of WEE activity.[295] Accumulated degree-days (defined as the sum of daily mean temperature minus the developmental threshold temperature) served as a predictor in the Rocky Mountain region.[130] Such data are readily obtained from the local weather service.

Vector data: Surveillance programs will measure relative vector densities based on CO_2-baited light trap or lard can trap collections, and will correlate light trap data with levels of WEE virus activity.[224] Pools of vector species sould be submitted for processing for virus isolation at a state or regional laboratory.

Vertebrate host data: Programs should sample wild and peridomestic passerine birds that are known or suspected to be locally important for enzootic or epizootic transmission.

Other data: There is some question regarding whether sentinel chickens provide sufficient lead time to react to the appearance of WEE virus. In some areas (e.g., Imperial County, California), high seroconversion rates are observed annually without the appearance of human or equine cases. Passive or active surveillance for equine cases may be useful, but reaction by health agencies must be rapid to have an impact on transmission once equine cases have been diagnosed.

CHAPTER 3
EASTERN EQUINE ENCEPHALOMYELITIS

Introduction

Enzootic transmission of EEE virus occurs regularly in freshwater swamp habitats along the Atlantic and Gulf Coasts of the U.S. Isolated foci occur in southern Michigan,[177] Ohio, and upstate New York[203] (Fig. 3-1). In Canada, EEE virus has been isolated occasionally in Ontario, Alberta, and Quebec.[6] During periods of intense transmission, the virus is dispersed from these foci by infected mosquitoes or viremic birds. These vectors or bird hosts initiate secondary transmission cycles outside the swamp habitat during the summer or early fall, which can lead to equine or human cases. EEE virus has been recovered in most other U.S. states east of the Mississippi River, although enzootic cycles are not known in those states.[202]

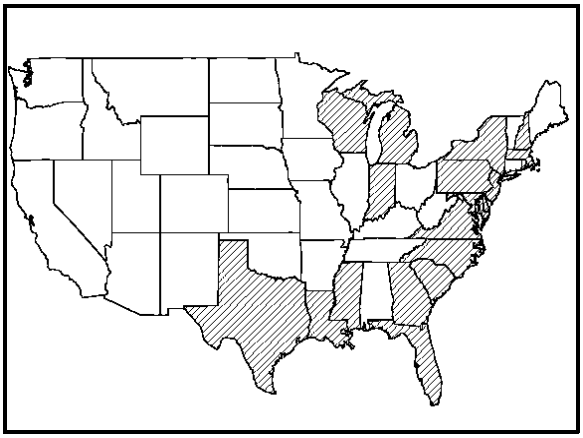

Figure 3-1. Distribution of confirmed and presumptive cases of eastern equine encephalomyelitis in the United States, 1964-1992.[c]

Epidemics of EEE are cyclic, with an interval between epidemics of about 9 years (Fig. 3-2). There seems to be no clear-cut relationship between epidemics and any known environmental factors. It is likely that a complex of environmental conditions must simultaneously impact on several parameters, such as vertebrate host population density, brood size and nutritional status, vector population density and longevity, and winter survival of both vectors and vertebrate hosts.

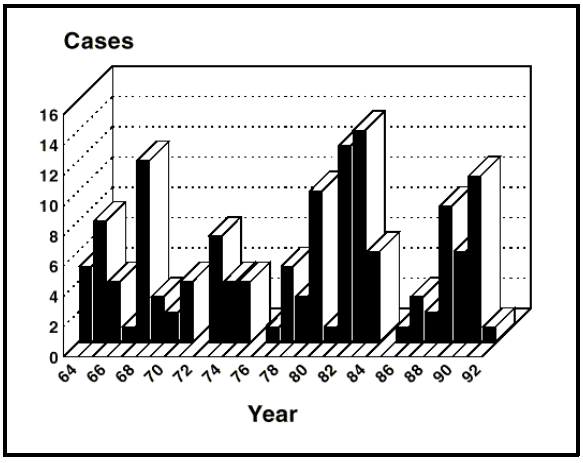

Figure 3-2. Reported cases of confirmed and presumptive human cases of eastern equine encephalomyelitis in the United States, 1964-1992.[c]

Meteorologic Data Monitoring

Rainfall patterns in Massachusetts and New Jersey have been associated with occurrence of EEE cases. Rainfall more than 20 cm above the average occurring in 2 consecutive years was associated with the beginning of 2-3 year cycles of human EEE outbreaks in Massachusetts.[106] The years 1930-1960 were ranked according to rainfall quantity in Massachusetts. There was an association between EEE outbreaks and years in which heavy rainfall occurred in June through August, preceded by heavy rains in August through October of the previous year. This correlation could not be established for other states. Hayes and Hess[124] analyzed weather patterns in relation to outbreaks of EEE. They concluded that heavy rainfall during the summer of an outbreak, combined with above average rainfall the preceding fall, produces a favorable environment for an epidemic. An unusually wet fall is probably conducive to successful overwintering of *Cs. melanura* larvae, and a wet spring facilitates rapid buildup of vector populations.

Letson[d] evaluated rainfall patterns in states and locales where human EEE cases occurred between 1983 and 1989. He found a significant association between the occurrence of human cases and excess rainfall in the year when cases occurred. The

[c] Tsai, T.F., P.S. Moore, and A.A. Marfin. Unpublished data.

[d] Letson, G.W. Unpublished data.

association was stronger with data from local weather stations than from statewide rainfall averages and the predictive model was best when applied to northern states. The sensitivity and specificity of these measures varied depending on the model used, but the positive predictive value was no more than 50% regardless of the rainfall model applied. Thus, although there appear to be significant associations between excess rainfall and epizootic EEE activity, a useful predictive model has been described only for Massachusetts.

In a retrospective analysis, the sporadic occurrence of human and equine EEE cases in certain northern states was traced by trajectory analysis to the northward movement of cold fronts carrying infected mosquitoes from more southerly locations.[257] The validity and possible predictive value of this hypothesis remains to be proven.

Vector Surveillance

A major question in the ecology of EEE is the identity of the bridging vectors that transfer the virus from the enzootic cycle to humans and equines. A variety of species serve as vectors, depending on time of year, environmental conditions, geographic location, and population dynamics.[120,254] These are discussed briefly below.

Aedes albopictus: (Asian tiger mosquito,[249] Forest day mosquito[281*,e]). *Aedes albopictus* is a recently-introduced mosquito native to Asia.[51,273] It has spread rapidly throughout the eastern U.S.[199,200] *Ae. albopictus* probably was introduced into the U.S. in shipments of used tires from Asia.[69,118]

In 1991, 14 isolates of EEE virus were obtained from 9,350 *Ae. albopictus* collected in Polk County, Florida.[53,191] The significance of this observation is unknown at present. *Aedes albopictus* has the potential to transmit other North American arboviruses, as well.[103,187,192,262]

The biology and behavior of *Ae. albopictus* is treated in detail in a recent review by Hawley.[117] This species oviposits readily in the CDC ovitrap. Adults respond to the duplex cone trap and to the CDC light trap baited with dry ice. Landing/biting collections, with or without additional dry ice attractant, are effective. Resting females can be collected with the Nasci aspirator or other large suction device (See Appendix II).

Aedes canadensis: (Woodland pool mosquito[281]). *Aedes canadensis* is widely distributed in the U.S. and Canada. A subspecies, *Ae. c. mathesoni*, is found in the southeastern U.S. EEE virus has been isolated from this species in New York.[137]

Larval habitats consist of woodland pools formed by melting snow or spring rains.[48] Larvae are most often found in pools with dead and decaying leaves on the bottom. Other larval habitats include roadside puddles, sink holes, wooded freshwater swamps, and isolated oxbows of small woodland streams. Adults of this species are abundant from March until October. There may be more than one generation per year.

Few estimates of daily survival have been attempted, but adults are said to live for several months.[48] In Newfoundland, where *Ae. canadensis* is univoltine, ovarian dissections confirmed the long life of this species. The gonotrophic cycle was estimated at 3 weeks, and 2-, 3-, and 4-parous females were estimated to have lived 6, 9, and 12 weeks respectively.[194] From these data the upper limit of daily survival can be estimated at 0.996 per day. The flight range of this species is reported to be short. Females rarely migrate far from larval habitats.[48] *Ae. canadensis* feeds primarily on mammals. In Maryland, 47% of bloodfed *Ae. canadensis* collected in the Pokomoke Cypress Swamp had fed on deer.[162] Interestingly, 16% of the females had fed on reptiles.

This species is readily collected in CDC and New Jersey light traps. Landing-biting collections are also effective.

Aedes sollicitans: (The salt marsh mosquito[281*]). *Ae. sollicitans* has been implicated as a bridging vector of EEE in New Jersey.[62,66] It may be an important vector in other parts of its range, as well. This species is common along the Atlantic and Gulf coastal plains, extending into Texas and Oklahoma. However, isolated population foci have been reported from brackish water in states as diverse as Arizona, North Dakota and Michigan.[71]

In coastal sites, *Ae. sollicitans* is associated

[e] Common names approved by the Entomological Society of America are indicated by '*'.

with salt-marsh grasses.[135] In Louisiana coastal marshes, saltgrass (*Distichlis spicata*) was the best predictor of *Ae. sollicitans* habitat.[102] In North Carolina coastal dredge sites, egg laying was associated with new stands of *Aster subulatus*.[255] Inland larval habitats have been associated with oil fields in various areas,[46] with sewage and high sulfate content in Michigan,[58] and with septic tank overflow plus road salt accumulation in western New York.[22]

Aedes sollicitans has 5-8 broods per year in New Jersey, and breeding is continuous in more southern areas such as Texas.[135] The eggs of some populations are photosensitive and enter diapause under short day conditions.[225]

During the day, adults rest on vegetation such as salt hay (*Spartina patens*) and saltgrass,[48,68] where they can be collected by vacuum aspiration. Adults are strong fliers and, during migratory flights, may fly as far as 64 km (40 mi) with wind assistance. A "large swarm" was once encountered by a ship 166 km (100 mi) east of coastal North Carolina.[135] They commonly disperse in large swarms from larval habitats in search of hosts, leaving about dusk, and may fly 5 to 10 miles in a single night. They are attracted to lights and thus to urban areas where they are a significant pest problem as well as potential vectors of EEE. Females return to marsh habitats to oviposit following the initial migratory flight. In New Jersey, parous females do not engage in repeated dispersal. They remain close to the marsh during later gonotrophic cycles, thereby concentrating potential human exposure in the marsh area.[67]

Aedes sollicitans females feed almost exclusively on mammals. In Florida, 97% of *Ae. sollicitans* females had fed on mammals, and 3% had fed on ciconiiform birds. Of the mammal feedings, 79% were on rabbits.[89] In New Jersey, 98% of blood meals came from mammals, with slightly more than 1% of meals from birds.[68] Deer were the most frequent mammalian host. In upland areas, avian hosts were most often passerine and gallinaceous birds, while in salt marsh areas virtually all meals came from ciconiiform birds. The low rate of feeding on birds may still be sufficient to account for the importance of *Ae. sollicitans* as an epizootic EEE vector given the high population density of this species.[68]

No direct estimates of survival appear to have been made for *Ae. sollicitans*. In New Jersey, 36.3%, 53.5%, 8.8% and 1.4% of females had completed 0, 1, 2 and 3 gonotrophic cycles, respectively.[87] This yields survival estimates of between 16.2% and 31.4% per gonotrophic cycle. Another study in the same area over a two-year period gave estimates of 30.4% and 50.6% survival per generation.[88] In Connecticut, a similar study found 53.9%, 37.1%, 9,0% and 0% of females had completed 0, 1, 2, and 3 cycles, leading to an estimate of 40.8% survival per gonotrophic cycle.[168]

Aedes sollicitans is readily collected in light traps, with and without CO_2. Resting females can be collected by vacuum aspiration or with a sweep net.[68] Large numbers of host-seeking females can be collected in landing-biting collections.[87]

Aedes vexans: (The inland floodwater mosquito,[136] vexans mosquito[281*]). EEE virus has been recovered from *Ae. vexans* in several states.[254] It is thought to be involved in the transmission of EEE to horses and humans in Massachusetts.

Aedes vexans is found throughout the Holarctic, Oriental and Pacific regions. In the New World, it is found throughout Canada and the U.S., extending southward through Mexico to Belize and Guatemala.[154,71] Adults appear in much of the U.S. in May, and are active through September.[136] Seasonal abundance is strongly affected by rainfall and flooding. Adults may disappear during long summer droughts.[136] (For an extensive review of the biology and behavior of this mosquito, see Horsfall et al.[136]).

Larvae are found in newly-flooded depressions created by river flooding, irrigation runoff, or rainfall. Specific sites include river flood plains, upland woods, wet prairies, ditches, canals and irrigated pasture.[136] Larvae usually can be found around the periphery of these habitats, particularly in the early instars.[136]

Newly-emerged adults rest in shrubs and grasses at the margins of the larval habitat. Later, they can be found in vegetation (grasses, flower beds, shrubs, etc.) in and near urban centers and farm buildings, or in livestock pastures and other areas where hosts may be found.[136] *Aedes vexans* engages in dispersal flights from larval habitats. Depending on wind conditions, adults may fly or be carried as much as 48 km (30 mi) from emergence sites.[136] Flight activity is almost entirely crepuscular.

Aedes vexans readily bites humans, and is a major pest species in the U.S. Although primarily a mammal feeder, this species also will feed on birds.[136,260] In host preference studies in several areas

of California, 60-66% of female *Ae. vexans* fed on mammals, with 10-13% feeding on humans.[243] In a Florida study, 99.5% of blood meals were from mammals. The primary hosts were ruminants, armadillos and rabbits.[89] In a study at rural and playa lake habitats in Hale County, Texas, 95% of blood meals were from mammals, with less than one percent of meals from humans. Host abundance varied between habitats. Forage ratios for domestic mammals were 12.1 and 10.0 at rural and playa lake habitats, respectively.[126]

Despite the importance and widespread abundance of *Ae. vexans*, daily survival has rarely been estimated for this species. Horsfall and associates estimated adult life at three weeks in summer and six weeks in spring.[136] In northern Colorado, daily survival between June and August was estimated at 0.665 by the apodeme banding method, and 0.688 by parity measurement.[193]

This species is readily collected by light traps, with or without CO_2. Power aspirators can be used to collect resting adults, and host-seeking adults can be collected in landing/biting collections.

Coquillettidia perturbans: (Irritating mosquito,[281] salt and pepper mosquito). EEE virus has been isolated frequently from *Cq. perturbans*. This species is believed to be an important bridging vector involved in transmission of the virus to equines.[254] In Florida, the minimum field infection rate (MFIR) for this species over a 20-year period was 1:34,980 (0.03 per 1,000).[30]

Coquillettidia perturbans occurs throughout most of the U.S. and southern Canada. It is absent or rare in the plains and southwestern states, but extends southward into Mexico along the Gulf coast.[71] This species normally has only one generation per year except in Florida, where there are two and occasionally even three generations.[48,167] In south Florida, adults of the first generation emerge in mid-March through mid-July. Those of the second generation emerge from mid-July to mid-October. In more northerly parts of the range, a single peak occurs between June and August.[2]

Coquillettidia perturbans larval habitats are freshwater marsh areas. The larvae attach to the submerged roots of aquatic plants by a specially adapted siphon. They are typically associated with cattails (*Typha* spp.), sedges (*Carex* spp.) and floating plants such as water hyacinth (*Pistia* spp.). In Florida, *Cq. perturbans* were found in significantly greater numbers where the bottom had a thick layer of detritus and in sites adjacent to wooded shorelines.[43]

Adults rest on leaves of grass and other low vegetation in cool, shaded locations during the day. Males may be especially abundant in grasses and rushes near the water.[135] The adults of *Cq. perturbans* are strong fliers, and will move several miles from larval habitats to surrounding populated areas to seek hosts.[135] They are readily attracted to CDC and New Jersey light traps, with or without CO_2. Swarming has been observed in Florida.[222] This species readily enters houses and bites humans.[135] Biting occurs mostly at dusk, with a second peak after midnight.[135] In shaded situations, females also will bite during the day.[31] In a Florida study, more than 90% of blooded *Cq. perturbans* females had fed on mammals. Most feeds were on ruminants (the most abundant hosts in the study area), while armadillos and rabbits were also well represented.[89]

Culex nigripalpus: (No common name[281]). EEE virus has been isolated from *Cx. nigripalpus* on a number of occasions. The significance of this species in the ecology of EEE has not been clearly established.[216] In Florida, the minimum field infection rate (MFIR) for this species over a 20-year period was 1:21,150 (0.05 per 1,000).[30] For a discussion of the biology of *Cx. nigripalpus*, see Chapter 5, SLE.

Culex salinarius: (Unbanded saltmarsh mosquito[281]). EEE virus has been isolated from *Cx. salinarius* in Florida, Alabama, South Carolina, Maryland and New Jersey.[254] The role of this species as an epizootic or epidemic vector is uncertain. This and several other species probably serve as vectors depending on time of year, environmental conditions, geographic location and dynamics of the vector populations.[254]

Culex salinarius occurs throughout most of the eastern United States, and is especially common along the Atlantic and Gulf Coasts. Despite its name, *Cx. salinarius* is not found predominantly in salt- or brackish-water habitats.[135] However, in coastal Louisiana, oviposition sites were associated with saltgrass stands.[102] Larval habitats consist of semi-permanent ponds, ditches, springs, seeps, and artificial containers.[135] Freshwater impoundments in coastal areas may generate large populations of this species.[268]

Adults can be found during the day in buildings, culverts, and similar cool, shaded sites. Overwintering adults have been collected in dwellings,[135] but not in animal burrows.[268] In New Jersey, adults begin to appear in light trap collections in May, with peak abundance in July.[266] Activity continues late into the fall, well after other species have entered diapause. Although fall collections are virtually all nulliparous, the first collections of adult females in the spring were more than 90% parous.[266] This could be a result of winter or early spring feeding, or a negative response to light traps before the first blood meal in overwintering females.

This species apparently engages in migratory flight, and unobstructed flights over water of 12.8 km (7.7 mi) have been reported in Delaware.[135] In Louisiana, marked females were recaptured 2 km (1.2 mi) from a release site within 26 hr after release.[160] The latter specimens were presumed to be engaging in host-seeking dispersal, since they were collected in CO_2-baited light traps.

Culex salinarius is a general feeder that feeds primarily on mammals in some habitats. In a study of two Florida localities, the ratio of bird to mammal feeding was 1.3:1 at one site and 1:19 at a second site.[90] In another study, populations from Minnesota were found to have fed primarily on passerine birds, while populations from Texas fed entirely on mammals.[284] This species feeds readily on hu mostly out-of-doors but occasionally inside buildings. Feeding is heaviest at dusk. In New Jersey, most host-seeking females were collected in the first two hours after sunset, but host-seeking activity continued through the night.[267] Adults may be collected from diurnal resting shelters or by use of light traps. Pigeon traps have also been used to collect this species.[267]

Culiseta melanura: (Blacktailed mosquito[281]). *Cs. melanura* is the primary enzootic vector of EEE in the U.S. In Florida, the MFIR for this species over a 20-year period was 1:1,825 (0.55 per 1,000).[30] Transovarial transmission of EEE in *Cs. melanura* has been suspected since several workers have reported virus in males[54] or in larvae.[122] However, later laboratory and field studies in New York,[205] Massachusetts,[122] and Maryland,[254,272] did not detect evidence of transovarial transmission.

This species occurs in the eastern United States from Canada to the Gulf of Mexico. It has been collected in all states east of the Mississippi River except Vermont and West Virginia. However, it is uncommon or rare throughout much of its range due to the lack of suitable larval habitats. Adult emergence begins in late May or early June in New York,[207] and in late April in Maryland.[147] Emergence is somewhat earlier in more southerly states. Oviposition occurs from mid- to late June through October. There may be 2, 3, or more adult emergence peaks during the season, depending on temperature and rainfall conditions. There are two summer generations and one overwintering generation in Maryland.[147] Adults are most numerous during late summer and early fall and persist until October. This species overwinters in the larval stage.[147]

Culiseta melanura larvae are most often found in heavily shaded sites associated with uprooted or decaying trees in permanent freshwater hardwood swamps.[147] These sites are frequently characterized by the presence of an interwoven root mat with a matrix of peaty soil.[210] Indicator tree species are red maple (*Acer rubrum)*, swamp white oak (*Quercus bicolor)* and white cedar (*Thuja occidentalis)* in northern states;[203] and with baldcypress (*Taxodium distichum*), sweetgum (*Liquidambar styraciflua*) and tupelo (*Nyssa aquatica*) in the southeastern U.S.[152,276] Although artificial containers do not constitute a primary habitat for this species, larvae have been found on several occasions in discarded tires.[251] Larvae also have been found in water in a concrete-lined pit in a utility tunnel[271] and in water collecting at the bottom of a resting box.[207]

Adult *Cs. melanura* can readily be found during the day in natural resting sites such as tree holes or fallen logs.[207] Adults seek daytime shelter both at the swamp edge and at upland "congregating sites" where they probably gather following host-seeking flights.[138]

Adult females are most active during the evening twilight period, but some activity continues throughout the night. Very little adult activity occurs during the daylight hours.[207] Mark-release-recapture studies in New York showed that *Cs. melanura* females moved a mean distance of 9 km (5.6 mi) from the release site. Thus, *Cs. melanura* may play an active role in transporting EEE virus to upland areas.[138] This may be particularly important when parous females make up a large percentage of the dispersing population.[215]

Host-seeking activity begins shortly after sunset, peaks within the first 2 hours after dark, and then continues at a relatively constant level throughout

the night.[214] *Culiseta melanura* feeds primarily on passeriform birds, feeding uniformly at heights between 1.5 and 7.6 m.[93,206,213] Other birds, mammals and reptiles are less frequent hosts.[147,206] Humans are rarely bitten.[123]

Little is known about survival rates of *Cs. melanura*. A single study in Massachusetts estimated daily survival at 0.749 to 0.814.[215] There is no apparent relationship between body size and either parity or infection with EEE virus,[166] as might be expected for a species with stable, nutrient-rich larval habitats.[101]

Adult *Cs. melanura* can be collected in both CDC and New Jersey light traps.[147,171] Adult females are also attracted to bird-baited traps, and can be collected from artificial resting shelters.[138] In one study, significantly more parous females were collected in CO_2-baited CDC light traps than in resting boxes.[207] As with most mosquito species, blooded females are rarely collected in either regular or CO_2-baited CDC light traps.[138,210] Resting boxes collect the largest numbers of blooded females.[147]

This species is usually very abundant in years in which EEE epizootics occur. Surveillance of *Cs. melanura* over a 5-year period in Connecticut, for example, noted a twelve-fold increase in the population during an EEE outbreak year.[294]

Vertebrate Host Surveillance

EEE virus activity is most intense in bird populations associated with fresh-water swamp forest habitats. These habitats are the foci for enzootic EEE virus transmission between bird hosts and *Cs. melanura* during the summer months in the northern states[70,98,121,177] and throughout the year in southern states.[275]

Virus or antibody have been detected in enzootic foci in many bird species, particularly passerines, although some species are more intensely involved than others. Some primary host species are the thrushes (wood, gray-cheeked, Swainson's, Hermit and Veery), catbird, cardinal, rufous-sided towhee, sparrows (song, swamp, white-throated), blue jay, vireos (red-eyed and white-eyed), Carolina wren, tufted titmouse, chickadees (Carolina and black-capped), warblers (Kentucky, black and white, yellowthroat and ovenbird), woodpeckers (downy and hairy), and flycatchers.

Once EEE virus leaves the swamp habitat via an infected mosquito or viremic bird, other bird species and equines may become involved. Some birds that regularly occur in both habitats and that could carry the virus between these habitats are the cardinal, common grackle, red-winged blackbird, American robin, song sparrow and blue jay. The post-reproductive flocking and random movement behavior of some of these species, particularly the more susceptible juvenile birds, may contribute to the dissemination of virus out of the swamp habitats. Recent studies in New Jersey indicate that the glossy ibis may function to move EEE virus out of swamp habitats. Post-reproductive ibises roost at night in the swamp forest and feed outside the swamp during the day.[f]

The wild birds that can function as amplifying hosts in mixed and agricultural habitats outside the swamps are the American robin, American goldfinch, barn swallow, house sparrow, cardinal, common grackle, starling, and red-winged blackbird.

Antibody prevalence in wild birds associated with well-established enzootic EEE foci in fresh-water swamps ranged from 6-85% in Alabama[275] and from 5-80% in Maryland.[70] For most of the primary species mentioned above, antibody prevalence averaged between 30-50%. During epizootics outside these "permanent foci", similar antibody prevalence rates in local wild bird populations were observed in Massachusetts[122], New York[98], New Jersey[274], and Michigan[177]. In Massachusetts and New York, the antibody prevalence in these same wild bird populations fell to <10% after 3 consecutive non-epizootic years.

Mortality from EEE virus infection occurs in wild birds in addition to the well-known mortality in ring-necked pheasants and other exotic game bird species.[202] The effect of this mortality on local bird populations must be considered when conducting surveillance using these species. However, some surveillance programs use captive ring-necked pheasants as sentinels and monitor the morbidity and mortality in this species as an indicator of EEE virus activity. Some examples of vertebrate species that have been used for surveillance of EEE virus activity are presented in Appendix III.

[f] Crans, W.J., Personal communication.

Gaps in current knowledge of eastern equine encephalitis

Answers to the following questions could greatly improve our understanding of and ability to predict, prevent, or control epidemic transmission of EEE. We suggest that, where possible, programs should collect data that could help to provide those answers. For additional information or assistance in designing studies of this type, consult your state health department, state vector control specialist, or contact the Division of Vector-Borne Infectious Diseases, Centers for Disease Control, Fort Collins, Colorado 80522.

- What is the overwintering mechanism of EEE virus?
- What is the relationship between weather patterns, *Cs. melanura* population density and EEE virus amplification patterns?
- Is there a usable relationship between degree-day accumulation and EEE virus amplification rates in the field?
- Which mosquito species are involved in epizootic transmission of EEE virus in different regions of the country?
- Which bird species are most important in EEE virus amplification?
- What is the relationship between EEE virus infection rates in the bird population and transmission of virus to mammals by bridge vectors?
- What is the role of *Ae. albopictus* in the ecology of EEE in the southeastern U.S.?
- What are the most reliable predictors for human risk of EEE infection?
- Are domestic animals other than horses (e.g., goats, pigs, cattle) useful as sentinels for monitoring epizootic EEE activity?
- What impact, if any, does EEE virus have on the dynamics of endangered or protected bird species other than the whooping crane?

CHAPTER 4
LA CROSSE AND RELATED CALIFORNIA SEROGROUP VIRUSES

Introduction

The California serogroup consists of several related viruses, some of which cause disease in humans. The association of California serogroup viruses with human illness was not apparent until the 1960's.[129,304] In North America, those California serogroup viruses known or suspected to cause human disease are California encephalitis (CE), trivittatus (TVT), snowshoe hare (SSH), La Crosse (LAC), and Jamestown Canyon (JC).[161] Figure 4-1 shows the reported distribution of human encephalitis cases due to California serogroup infections. This document will discuss only LAC, CE and JC viruses.

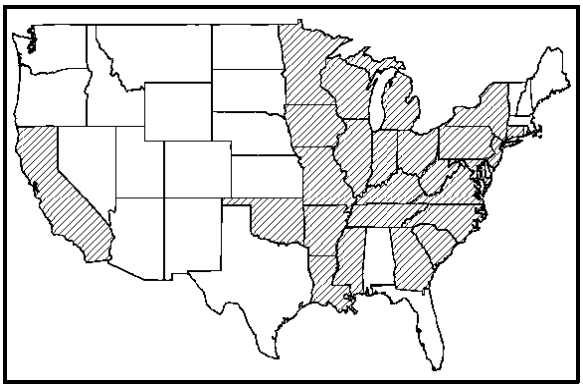

Figure 4-1. Geographic distribution of confirmed and presumptive human cases of California serogroup encephalitis (LAC, JC, CE) in the United States, 1964-1992.[g]

Transmission of California serogroup viruses, including LAC, JC, and CE, to humans is rather constant when compared to other arboviral encephalitides (Fig. 4-2). There are about 75 reported cases nationally (range 30-160) each year.[50,148] This relative constancy may be because transovarial transmission plays such a major role in virus maintenance. Thus, year to year changes in vertebrate host densities may have little impact on the level of virus activity in vector mosquitoes. The ecology of LAC virus has been studied extensively in Wisconsin,[305] New York[108] and Ohio[24]. Its ecology is unique and reasonably well defined. The principal vector is a tree-hole breeding mosquito, *Aedes triseriatus,* and the major mammalian hosts are the eastern chipmunk, tree squirrels and foxes.[305]

The natural LAC cycle occurs in numerous woodland habitats and isolated woodlots in the north central states. Transovarial transmission plays an important role in the maintenance cycle of LAC virus.

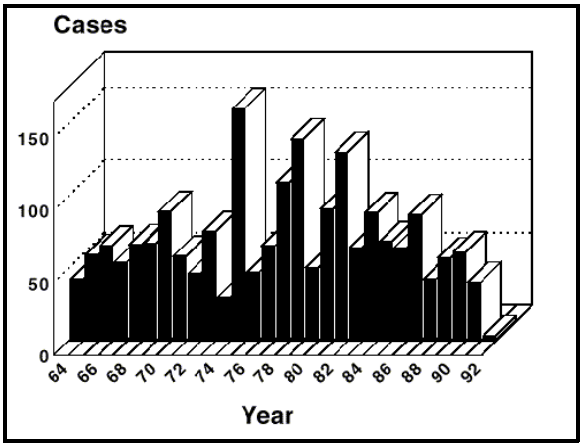

Figure 4-2. Reported confirmed and presumptive cases of encephalitis in humans due to viruses of the California serogroup (LAC, JC, CE) in the United States, 1964-1992.[g]

Jamestown Canyon virus produces moderate to severe involvement of the central nervous system.[79] Since most state laboratories do not specifically test for JC virus, it is difficult to estimate the annual incidence of JC virus infection. However, a serosurvey of Michigan residents found neutralizing antibody to JC virus in 27.7% of 780 individuals sampled.[110] JC virus infections differ from LAC virus infections; clinical illness occurs more often in adults, and meningitis is more common than encephalitis.[79]

The ecology of JC virus differs from that of LAC virus. The primary mammalian host is the white-tailed deer (*Odocoileus virginianus*).[141,218] JC virus does not produce a viremia in rabbits or squirrels.[78] Although JC virus was first isolated from *Culiseta inornata,*[149] most JC virus isolates have come from various *Aedes* species including the *Ae. communis* group,[44,108,161,280] (primarily *Ae. provocans* in New York[32] and Michigan[127], but *Ae. abserratus* in Connecticut[170]), *Ae. stimulans,*[33,280] and *Ae. excrucians*.[108] Although isolates of JC virus from *Anopheles* species are uncommon,[24,108] anophelines are

[g] Tsai, T.F., P.S. Moore, and A.A. Marfin. Unpublished data.

proposed as early season vectors of JC virus.[78]

California encephalitis (CE) virus causes infection in humans, but clinical disease apparently is rare.[109,233] The natural cycle of CE virus probably involves *Aedes* species, particularly *Ae. melanimon* and *Ae. dorsalis*, and small mammals such as the California ground squirrel, *Spermophilus beecheyi*.[161] Transovarial transmission in *Ae. dorsalis* is a possible overwintering mechanism for CE virus.[60,161] Laboratory studies suggest that subpopulations of *Ae. dorsalis* may develop stabilized infections, transmitting CE virus to more than 90% of their offspring.[291]

Meteorologic Data Monitoring

Larval development of the LAC vector, *Ae. triseriatus*, is dependent on natural and artificial container habitats that are filled primarily by rain water. Thus, variation in rainfall has a definite impact on vector density. Year-to-year variation in rainfall drastically affects the available number of container habitats.[263] Whether this affects the dynamics of LAC virus transmission still must be demonstrated.

Vector Surveillance

Aedes canadensis: (Woodland pool mosquito[281]). LAC virus is isolated regularly from *Ae. canadensis*, particularly in Ohio.[23] Low isolation rates from this mosquito in other areas may be due to differences in susceptibility to the three different subtypes of LAC virus, which have differing geographic distributions.[78] For a discussion of the biology of *Ae. canadensis*, see Chapter 3, EEE.

Aedes communis: (Common snowwater mosquito[281]). JC virus is frequently isolated from this mosquito. Pooled data from several surveys and studies suggest a minimum infection rate of about 1:1,538 for *Ae. communis* and related species.[96] This species occurs in deciduous and evergreen forests across the northern U.S., Canada, Alaska, Siberia, and northern Europe.[46] *Ae. communis* is a univoltine, woodland species, whose larval habitats are pools filled by melting snow. It is most abundant in the spring and early summer. Large mammals are the preferred hosts, and humans are readily bitten. Peak biting activity occurs after sunset, but females are reported biting throughout the day in shaded locations.[46] Adults are long-lived; the daily survival rate of *Ae. communis* in the Sierra Nevada of California is estimated at 0.88 - 0.91.[96]

Aedes dorsalis: (No common name). CE virus is isolated from *Ae. dorsalis*, particularly in Utah. CE virus is passed transovarially in this species,[60] in which stabilized infections can result in vertical transmission rates of more than 90%.[291]

Ae. dorsalis is a holarctic species. In North America it extends from about 55°N in western Canada to about 50°N in eastern Canada, southward to the Mexico border in the western U.S. *Ae. dorsalis* is absent from the southeastern U.S.[71] This mosquito occurs in a variety of habitats. Larval habitats include tidal marshes along the Pacific coast and saline pools associated with the Great Salt Lake in Utah.[46] Other larval habitats include fresh-water marshes and roadside ditches. Grassy, sunlit habitats are preferred.[46] In Manitoba, larvae were most frequent in temporary pools located near blood meal sources of the adults.[81]

Eggs hatch after being flooded in the spring, and there can be several generations each year. *Ae. dorsalis* is an important pest species in some areas. Females are vicious biters, with the bulk of host-seeking activity in the evening,[46] although they also will attack during daylight hours. Dispersal flights of 20 - 30 miles are recorded.[46] Large mammals usually are the preferred hosts of *Ae. dorsalis*,[81,243] but 46% of blooded *Ae. dorsalis* collected in western Utah had fed on rabbits.[61] The length of the first gonotrophic cycle was about 5 days during July - August in northern California, and estimated survival was 14% per gonotrophic cycle (67% per day).[146] Adults of *Ae. dorsalis* are collected in large numbers in CO_2-baited light traps.[146]

Aedes melanimon: (No common name). California encephalitis (CE) virus is maintained through vertical transmission by infected clones of *Aedes melanimon.* In the Sacramento and San Joaquin valleys of California, horizontal transmission to jackrabbits amplifies the virus in the summer .[235,243] CE is not a common cause of encephalitis in humans in California. Reeves[233] found evidence for CE infection in only 18 of 1,637 (1.1%) paired sera collected between 1965 and 1976 from patients with febrile and CNS illness in that state. See Chapter 6 (WEE) for a review of the biology of *Ae. melanimon.*

Aedes stimulans: (Brown woods mosquito[281]). *Ae. stimulans* is a common host of Jamestown Canyon (JC) virus. Isolation of JC virus from larvae and males of this species suggests a possible role of *Ae. stimulans* in transovarial

maintenance of the virus.[78,96] *Ae. stimulans* is a common mosquito in the northeastern and midwestern states, extending westward into North and South Dakota, Nebraska, and Kansas. In Canada, it occurs in southwestern Manitoba, southern Quebec, New Brunswick, Nova Scotia, and Newfoundland.[71] The distribution of *Ae. stimulans* roughly matches the distribution of northern floodplain forests (deciduous, transition, evergreen) in the U.S.[21] Larval habitats of this woodland species consist of temporary pools formed by melting snow, spring flooding, or spring rains.[46]

Ae. stimulans is an early season species. Adults are found as early as April or May, depending on locality and temperature.[33,46] *Ae. stimulans* will seek a blood meal at all hours within the shade. While it feeds primarily on deer,[33] *Ae. stimulans* also is a persistent biter of humans and a major pest in some areas.[46] *Ae. stimulans* females were attracted to and fed on chickens, woodcock, and domestic rabbit in studies using caged bait animals.[303] CO_2-baited light traps[33] or small Magoon traps with bait animals readily attract *Ae. stimulans*.[303] Resting adults can be collected by using large, battery-powered aspirators.[33]

Aedes triseriatus: (The eastern treehole mosquito). *Aedes triseriatus* is the primary vector of LAC encephalitis virus. LAC virus is vertically transmitted in this species.[292,298] Vertical transmission provides an efficient overwintering mechanism for the virus.[75,299] LAC virus foci often are highly stable over time. In a 4-year Illinois study, 14 of 50 treeholes contained transovarially-infected larvae. One of the trees was positive in 3 of the 4 years.[56] There is a strong association between the occurrence of LAC encephalitis cases and the presence of *Ae. triseriatus* in artificial containers, such as tires, on patients' premises.[59,128]

Aedes triseriatus occurs in hardwood forest areas of North America east of about 100º W longitude, from northern Mexico to southern Canada.[135,306] The appearance of adults in the spring is strongly dependent on temperature in the larval environment, and probably also on available nutrients. In an Indiana study, pupae appeared about 2 weeks earlier in tires exposed to full sun than in shaded tires, and about 4 weeks earlier than in treeholes. Treeholes were the coolest of the three habitat types.[114] Multiple emergence peaks during the season are associated with rainfall events.

The larvae of this species develop in rot holes in deciduous trees, and in artificial containers of all kinds. Discarded tires are a frequent source of large *Ae. triseriatus* populations. Occasionally, larvae occur in rockholes.[306] Where *Ae. triseriatus* and *Ae. hendersoni* overlap, *Ae. triseriatus* larvae are more common in treeholes near the ground.[264]

Adults rest in shaded locations during the day. They often remain near larval habitats, particularly in wooded sites,[135] but will fly into open areas to feed.[76] *Aedes triseriatus* does not appear to have a migratory flight. Dispersal is more often along fence rows rather than across open areas. Most flight activity occurs during the early morning and late afternoon hours, a result of host-seeking activity.[9] *Aedes triseriatus* females feed almost exclusively on mammals, including humans. Preferred hosts include chipmunks, squirrels and deer.[36,212] In North Carolina, however, the majority (75%) of blood meals taken by *Ae. triseriatus* were from reptiles or amphibians.[140]

Several estimates of adult longevity are available. In Indiana, mark-release-recapture studies gave estimates of daily survival ranging from 0.78[230] to 0.96.[293] An Ohio mark-release-recapture study obtained estimates of 0.93 to 0.97 per day.[115] Several factors, including temperature, humidity, and larval nutrition, affect adult survival rates.[159,293]

Several traps are available for *Ae. triseriatus*, but none are totally satisfactory.[59] Although *Ae. triseriatus* is a diurnal species, it enters light traps in small numbers. Adults are reluctant to enter bait traps. Landing/biting collections are expensive, time consuming, and expose collectors to possible infection by LAC virus.[158] Large, battery-powered suction devices collect sizeable numbers of adults,[211] but this also is a laborious and time-consuming operation. A CO_2-baited, modified Pfuntner trap was significantly more attractive than mouse-baited or un-baited traps, but no trap collected more than 37 females per day.[158]

Oviposition activity of *Ae. triseriatus* is monitored by using ovitraps. This method also provides estimates of vertical transmission of LAC virus.[164] Trap color, substrate texture, position of opening, optical density of water, and the presence of organic decay products affect trap efficiency.[164,300] Several compounds of tree or larval origin are attractive to ovipositing females.[19,20] Fish oil emulsion has produced mixed results as an oviposition attractant for *Ae. triseriatus*.[16,131]

Culiseta inornata: (No common name). In the western U.S., *Cs. inornata* is considered an

important vector of Jamestown Canyon virus and it's variant, Jerry Slough (JS) virus.[161,243] *Cs. inornata* is a widespread species. It occurs from Florida to New Hampshire in the east; in the west, it occurs from northern Mexico to the Yukon and Northwest Territories.[46,71] In California, this species occurs in coastal marsh, agricultural, desert, Sierra foothills habitats.[243] Larvae can tolerate high concentrations of mono- and bi-valent salts, which allows them to exploit saline and alkaline habitats as well as fresh water habitats.[243] In Utah, the water temperature of pools with *Cs. inornata* larvae averaged from 2° to 5° F cooler than pools with *Cx. tarsalis, Cx. pipiens*, and *Ae. dorsalis*.[107]

In California, there is a bimodal pattern of seasonal abundance, with the major peak in October-November and a second peak in January-February.[243] Adults rarely appear in traps or in shelters during the summer, apparently because females enter a temperature and photoperiod-induced aestivation.[12] The appearance of males in resting sites in October signals the emergence of the progeny of aestivating females.[296] Temperature limits flight activity, with most activity occurring between 9° and 18° C.[183] In the Coachella Valley of southern California, a December study of biting activity found peaks of activity at dusk and around midnight. A second study in March found only a peak at dusk.[11]

Cs. inornata females prefer large mammal hosts, particularly cattle and horses.[4,296] Blood meals from birds are rare in nature.[243] However, *Cs. inornata* fed equally on both a rabbit and a chicken when the two hosts were placed together in a stable trap.[220] Autogeny occurs in *Cs. inornata*, and is temperature-dependent. The percentage of females with autogenous egg development may approach 30% at temperatures around 5° C.[183] The presence of summer aestivation makes estimating survivorship difficult. In California, estimates of seasonal parity differed over a two-year study period. In a marsh habitat, 2-5% of females completed two or more gonotrophic cycles, and 0.3-0.9% had completed three or more cycles. At a Sierra foothills site, 0-1.4% completed two cycles, and none completed three or more cycles in either year.[183]

This species is collected in small numbers in artificial or natural resting shelters.[183,296] CO_2-baited light traps readily collect *Cs. inornata*. In the Coachella Valley of California, New Jersey light traps collected three times as many *Cs. inornata* as sweeping with a D-Vac sweeper, 20 times as many as diurnal resting boxes, and 40 times as many as a suction trap.[12]

Vertebrate Host Surveillance

Maintenance and overwintering of LAC virus in nature is by transovarial transmission (TOT) of the virus *Ae. triseriatus*. Mammal hosts participate in the cycle by amplifying the virus and expanding the infection rate of the vector mosquito population during the summer months.

Some woodlots may contain virus-infected mosquitoes or hosts, while other woodlots nearby may be negative. The eastern chipmunk and tree squirrels are the major amplifying rodent hosts within the infected woodlots. Antibody prevalences in these species can reach nearly 100% by the end of the transmission season in September.[305] Mice and other rodents, cottontail rabbits, raccoons and opossums are much less frequently infected with LAC virus, though many are susceptible to experimental infection.

On the other hand, the infection rates in red and gray foxes have a temporal and spatial pattern similar to that of the chipmunks and human cases. Foxes within hyper-enzootic foci may have antibody prevalences as high as 68% compared to 18% outside of this area.[305] Not only are red foxes susceptible to infection by mosquito bite, but they also can acquire infection and become viremic by eating infected chipmunks. Infected foxes may help to spread the virus between isolated woodlots. The ecology of LAC virus may differ in areas peripheral to the north central states, particularly in the Appalachian region.

In the north central states (e.g., Indiana, Michigan, New York) Jamestown Canyon (JC) virus causes human disease.[109] The natural vertebrate hosts of JC virus are white-tailed deer in the eastern U.S.,[109,297] and mule deer in the western U.S.[45] These animals can be used to monitor the distribution and intensity of virus activity. Ground squirrels, jackrabbits, and cottontails are the natural vertebrate hosts of CE virus.[109,161]

Gaps in current knowledge of LAC and other California serogroup viruses

Answers to the following questions could improve our understanding of and ability to predict, prevent, or control epidemic transmission of LAC and other CAL serogroup viruses. We suggest that, where possible, programs should collect data that could help to provide those answers. For additional information or help in designing studies of this type, consult your state health department, state vector control specialist, or contact the Division of Vector-Borne Infectious Diseases, Centers for Disease Control, Fort Collins,

Colorado 80522.

- What are the most reliable predictors for human risk?

- What is the influence of rainfall and temperature on *Ae. triseriatus* population density and the amplification of LAC virus in a woodlot focus?

- What is the relationship between mosquito population density, vertebrate host density and LAC virus amplification?

- Do the relative densities of amplification hosts and non-amplifiers (i.e., large mammals such as deer) influence the status of LAC virus in a wooded area?

- What is the potential for *Ae. albopictus* to become involved in the transmission of LAC virus?

- What is the geographic distribution of LAC, JC, and other California serogroup viruses in the U.S.?

CHAPTER 5
ST. LOUIS ENCEPHALITIS

Introduction

SLE virus occurs throughout much of the U.S. (Fig. 5-1). It extends northward into Canada and southward into Central and South America in a variety of habitats.[288] SLE probably is not endemic to Canada, but periodically crosses the border as an extension of activity in the central and western U.S.[6] The ecology of SLE involves a wild bird-*Culex tarsalis* cycle in irrigated regions of the western U.S. It involves wild birds and members of the *Cx. pipiens* complex in the midwest and the east. Transmission in Florida is by *Cx. nigripalpus* mosquitoes, with birds and possibly mammals[176] as the primary vertebrate hosts.[288]

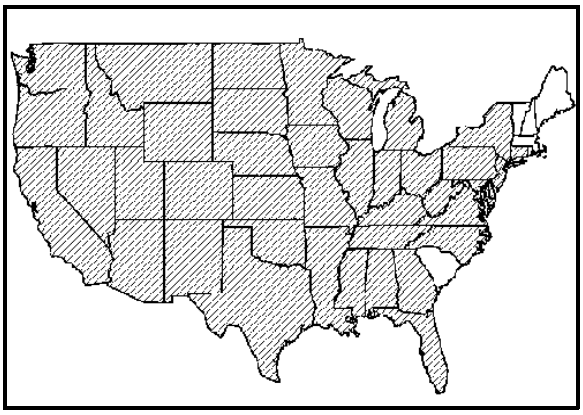

Figure 5-1. Geographic distribution of confirmed and presumptive human cases of St. Louis encephalitis in the United States, 1964-1992.[h]

Epidemics of SLE recur at irregular intervals or from 10 to 20 years (Fig. 5-2) For human cases reported for the period 1955 through 1992, autocorrelation analysis shows a recurrence of major activity approximately every 19 years. Reiter[247] has discussed several climatic factors that could lead to cyclic recrudescence of viruses such as SLE (Also, see below).

Meteorologic Data Monitoring

Meteorologic factors that have been shown to correlate with epidemics of SLE include rainfall and temperature as well as more general indices.

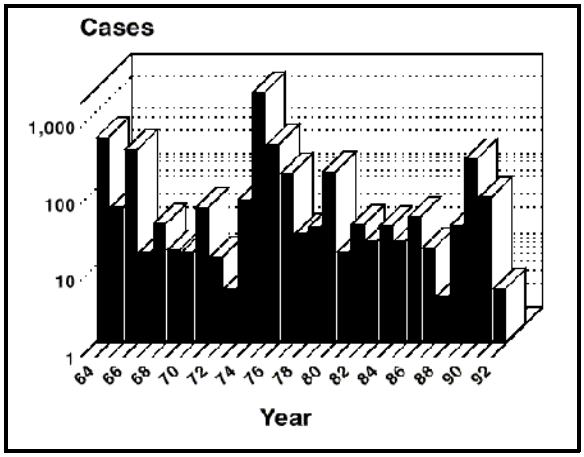

Figure 5-2. Reported cases of confirmed and presumptive human cases of St. Louis encephalitis in the United States, 1964-1992.[h]

The decennial cycle of urban SLE epidemics from the 1930s to the 1970s is correlated roughly with the inverse of sunspot activity.[i] SLE epidemics matched the 11 year sunspot cycle during this period except in the 1940s when no epidemics were reported. Personnel shortages during the Second World War may have reduced the sensitivity of disease surveillance during that decade. Sites of SLE outbreaks lie principally at southern latitudes below the 21° C isotherm for mean June temperature.[130] Numerous exceptions to this observation have been noted, including Chicago, Detroit, Ontario, Cleveland in 1975, and the Yakima Valley from 1939-42. However, unusually warm summer weather occurred in these northern locations in the epidemic years.

***Culex pipiens*-borne St Louis encephalitis**: Monath[196] reviewed monthly temperature and precipitation for 15 epidemic years and 30 non-epidemic years in 12 sites where SLE outbreaks had occurred. He used the criteria of deviation from the mean monthly precipitation or temperature at the epidemic site. Three significant differences were observed in epidemic versus non-epidemic years: 1) above average precipitation and temperature in January, 2) below average temperature in April, and 3) above average temperature in May. The strength of these associations varied regionally and the correlation

[h] Tsai, T.F., P.S. Moore, and A.A. Marfin. Unpublished data.

[i] Tsai, T.F. and E.D. Walker, Unpublished observations.

of monthly temperature with epidemic years was strongest for northern locations. Anecdotal observations have noted that epidemics frequently occurred after a hot dry summer. However, there was no significant association between temperature and precipitation indices in summer months and epidemic risk.

Several deficiencies in the foregoing study are noted here as a guide to planning future studies. Although there was a temporal control (i.e., epidemic and non-epidemic year), there was no spatial control (i.e., otherwise similar areas that had no SLE in either epidemic or non-epidemic years). The model was not applied to other locations in the Ohio-Mississippi valley where SLE potentially could occur. With so many other weather stations in this region it is improbable that the predictive value of this combination of indices could be high. Furthermore, the model was never validated. It should be applied to weather data from 1975-1990 for the specific sites that were examined in developing the model.

***Culex tarsalis*-borne St Louis encephalitis**: An analysis of California data from 1953-1973 found that both SLE and WEE incidence were associated with increased cumulative precipitation from January to July, and with above average mean monthly temperatures for April through June.[223] A study of the influence of springtime temperature on SLE and WEE transmission in northern Colorado revealed the accumulation of 10 degree-days above 75°F before the second week of June was associated with maximal seroconversion rates to SLE (but not to WEE) in sentinel chickens.[130] This association held only for northern latitudes.

Vector Surveillance

Extensive information on the biology, behavior and control of SLE vectors is available in separate publications.[35,47,189,236]

Culex restuans: (White dotted mosquito[281]). *Culex restuans* is similar in appearance and habits to the *Cx. pipiens* complex. However, it is usually unimportant as a pest and is more rural in occurrence. This species is widely distributed east of the Rocky Mountains from the Gulf of Mexico into Canada. It has been reported from all of the contiguous 48 states except Washington and Nevada.[189]

In 1975, SLE virus was isolated from *Cx. restuans* in Tennessee and Illinois,[189,195] and in the laboratory, *Cx. restuans* is an efficient vector of SLE.[55] However, the role of this species as either an enzootic or epizootic vector is still uncertain.[288] The early-season abundance of this species and the isolation of SLE from specimens collected in mid-May suggested it might be involved in enzootic amplification or overwintering.[189] However, long-term studies in Memphis, Tennessee, did not clearly demonstrate such a role.[197] *Culex restuans* appears early in the season and continues breeding in cooler areas throughout the summer. In warm areas, such as Memphis, adults are rare in mid-summer. They become abundant again in the fall when temperatures drop.[247]

Larval habitats are similar to those of the *Cx. pipiens* complex, i.e., ground pools or container habitats with high organic content. Larvae also can be found in rot holes in trees, rain barrels and discarded tires.[135,189]

Adults probably rest in grass, shrubs, animal burrows or other cool, humid sites during the day. They also can occasionally be found resting in poultry sheds and other animal shelters.[135] Adults overwinter in protected sites such as stone basements, mine shafts, natural and artificial stone caves, and stone outbuildings.[189] Little is known about dispersal and flight activity of this species. One study reported flights of at least 5.1 km over open water.[135]

Culex restuans is thought to feed primarily on birds.[135] More than 70% of over 500 blooded females collected in Minnesota and Illinois had fed on passeriform birds.[284] In a study of host feeding patterns of Florida *Culex* species, only two blooded *Cx. restuans* females were collected. One had fed on a bird and one on a mammal.[90] *Culex restuans* is variously reported as an annoying pest or as rarely biting humans. Much of the confusion is undoubtedly related to the difficulty of distinguishing adult *Cx. restuans* from adult *Cx. pipiens*. At best, this species is an occasional feeder on humans. Feeding is usually out-of-doors beginning at dusk and continuing sporadically through the night.

Adults are attracted to light traps, and they may be collected from sheltered resting places in the daytime.[248] They are readily collected in the CDC gravid trap[248] or oviposition pans. The population size can accurately be estimated in the presence of other *Culex* species by looking at first instar larvae.[245]

Culex salinarius: (Unbanded saltmarsh mosquito[281]). SLE virus is frequently isolated from

Cx. salinarius in the field.[57,189,195,197] However, the significance of this species as an epizootic or epidemic vector is not well defined.[288] Transovarial transmission of SLE virus by orally infected *Cx. salinarius* has been demonstrated in the laboratory.[217] For information on the biology of *Culex salinarius,* see Chapter 3, EEE.

Culex nigripalpus: (No common name[281]). *Cx. nigripalpus* is highly susceptible to SLE virus, and nearly all infected females transmit the virus under laboratory conditions.[278] It is the primary vector of SLE in Florida.[73,283]

This neotropical mosquito ranges northward from northern South America. *Cx. nigripalpus* is found in the U.S. from eastern Texas to the Atlantic coast and northward through Tennessee and North Carolina. It extends up the Mississippi-Ohio River basin to southern Indiana.[71] The species is particularly common in central and southern Florida, where it replaces the related species, *Cx. salinarius*. Elsewhere in its U.S. range, it is usually of scattered or rare occurrence.

Larval habitats consist of more-or-less permanent bodies of water such as ditches, grassy pools and catch basins. Occasionally, *Cx. nigripalpus* larvae can be found in artificial containers such as tires, and children's wading pools. During the day, adults can be found concentrated in areas of dense vegetation, such as oak or cypress hammocks.[216]

In Florida, *Cx. nigripalpus* has 8 to 10 generations per year, with as many as 15 broods.[216] Peak abundance is normally between August and December. The number of broods as well as oviposition and blood-feeding activity are strongly related to rainfall.[74,228] Females of this species can retain their eggs for extended periods. They oviposit only after rainfall of 51 mm or greater.[74] Recurrent patterns of heavy rainfall punctuated by extended dry periods lead to synchronization of oviposition and blood-feeding.[73,228] Synchronized feeding by many vectors could create temporal waves of infection in birds and mosquitoes. Such non-homogeneous mixing is expected, on theoretical grounds, to alter the basic dynamics of disease transmission.[85]

The dispersal and flight activity of this species have been extensively studied, but little work has been done to establish the maximum flight range. One study found that marked females dispersed at least 5 km (3 mi) from the release site.[82] Flight activity of *Cx. nigripalpus* (and probably many other species) is strongly affected by such factors as rainfall, humidity and wind speed.[27,83] *Culex nigripalpus* is primarily restricted to forest habitats, even at night.[26] During periods of heavy rain, however, host-seeking females will leave the forest habitat for open areas, which may influence host selection (see below).[90,91]

Culex nigripalpus is an opportunistic feeder on a variety of mammals and birds.[90,216] A seasonal shift in host selection has been demonstrated for this species in Florida.[90,91] Avian hosts (mainly Galliformes and Ciconiiformes) predominate in winter and spring. In summer and fall, there is equal or greater feeding on mammalian hosts. This shift is thought to be due primarily to higher summer and fall evening humidity, although defensive behavior by avian hosts may also be a significant factor.[90,92] Blood-feeding activity is correlated with daily rainfall, especially when rainy periods are separated by several weeks of drought.[72] *Culex nigripalpus* is less inclined to attack humans than is *Cx. salinarius*, particularly in winter and spring. Although females feed primarily at night, feeding on humans has been observed in the daytime in shaded hammocks in Florida.

Daily survival rates of *Cx. nigripalpus* in nature have been estimated to be as high as 0.81.[82] Daily survival ranged from a low of 0.66 in August to a high of 0.79 in September in a seasonal study in central Florida.[216] Higher survival rates were associated with moderate night temperature and higher humidity.

Adults are attracted to CO_2-baited CDC light traps, but do not respond well to New Jersey light traps. *Culex nigripalpus* can be collected readily with chicken-baited lard can traps.[216] Traps collect the most specimens when placed within forested areas rather than at the edge or in the open.[26] A greater proportion (but not a greater absolute number) of *Cx. nigripalpus* females collected in open fields are gravid. There is no difference in the proportion of parous females between wooded and open trap sites.[28] This species is occasionally collected inside houses.

***Culex pipiens* complex**: *Cx. pipiens pipiens* (the northern house mosquito[*281]) and *Cx. pipiens quinquefasciatus* (the southern house mosquito[*281]) are considered here as closely related subspecies because they are difficult to separate and crossbreeding is common. Some authors, however, consider them to be distinct species.[j,265]

Members of the *Cx. pipiens* complex are important vectors in urban epidemics of SLE, particularly in the midwest and Texas. *Culex pipiens* may have been an accessory vector in a 1985 SLE outbreak in western Colorado.[290] The two subspecies differ in their competence as SLE vectors in the laboratory. SLE virus develops more rapidly and to higher titers in *Cx. p. pipiens*.[55]

This group of domesticated species is found throughout the world.[154] In the U.S., *Cx. p. pipiens* occurs throughout the northern United States. It is found as far south as Georgia and Oklahoma. *Culex p. quinquefasciatus* occurs in all southern States. Hybridization between subspecies occurs in areas where their ranges overlap, as in Memphis, Tennessee.[13,143,229] These mosquitoes are the most common human-biting species in many urban and rural communities of the eastern U.S.

Larvae are usually found in water of high organic content, such as cesspools, dairy drains, and sewage lagoons, but also can be found in clean water. Population densities are highest in the dry season as water evaporates and organic concentration increases. The physical characteristics of larval habitats vary from roadside ditches, construction sites and ponds to artificial containers such as abandoned swimming pools, rain barrels, tin cans, and similar structures.[14,46] Discarded tires are a major source of *Cx. pipiens* complex larvae in urban areas.[5,15,199]

Adults can be found during the day in dark, damp shelters such as culverts, storm sewers, cellars, outhouses, and chicken houses,[135] where they can be collected by using mechanical aspirators (see below). There are several to many generations per year, depending on local climatic conditions. Anautogenous populations of *Cx. p. pipiens* enter winter diapause, while *Cx. p. quinquefasciatus* does not. There is some question about the ability of autogenous *Cx. p. pipiens* to enter diapause.[31] Females of *Cx. p. pipiens* do not take a blood meal before entering diapause.

Flight activity occurs mainly at night. In southern California, marked *Cx. p. quinquefasciatus* females traveled 0.91 km in 12 hr and 1.27 km in 36 hr.[253] In a nearby area, *Cx. p. quinquefasciatus* dispersal was related to host-seeking, and females were estimated to fly between 0.6 and 1.0 km/day.[240] The mean distance dispersed was lower in residential areas than in agricultural or park habitats.

Feeding is usually restricted to hours of darkness, peaking in periods of changing light intensity at dusk and dawn. Feeding activity in U.S. populations begins shortly after sunset, and most feeding is completed by midnight.[135] In Texas, however, a significant proportion of *Cx. p. quinquefasciatus* females fed between midnight and dawn.[119] A marked decline in feeding activity of *Cx. p. quinquefasciatus* occurred 2-3 hr before dawn in rural Kern Co., California,.[243]

In the U.S., females of the *Cx. pipiens* complex differ somewhat in their host-preference. Females of *Cx. p. pipiens* feed primarily on birds, and while *Cx. p. quinquefasciatus* females show a preference for avian blood, they readily feed on mammals including humans.[284] Feeding occurs inside or outside of dwellings.

The lack of definitive estimates of the length of the gonotrophic cycle under field conditions has prevented accurate estimates of survival based on parity.[243] Parity estimates in California ranged from 19% to 53%, with lower estimates near known emergence sites and highest estimates among host-seeking females.[243] Survivorship estimates of *Cx. p. quinquefasciatus* in southern California, based on mark-recapture data, ranged from 0.65 to 0.84 (65% to 84%) per day.[240] The apodeme banding method[252] was used to estimate survival in *Cx. p. quinquefasciatus* with limited success.[201]

Cx. p. pipiens are more readily attracted to light traps than are *Cx. p. quinquefasciatus*.[301] Neither subspecies is as strongly attracted to light traps as they are to chicken-baited cone traps.[34] In California, CO_2-baited light traps were more effective than New Jersey light traps.[243] Diurnal resting places offer convenient collecting sites, using hand or back-pack aspirators,[248] but this is an extremely labor-intensive activity. The CDC gravid trap[244,246] provides an effective and economical sampling system for members of the *Cx. pipiens* complex. Because this trap only collects gravid females seeking an oviposition site, a high percentage of females have fed at least once and the chance of isolating viruses is greatly increased.[248] In a California study, the gravid trap was only slightly more effective at collecting gravid and parous *Cx. p. quinquefasciatus* when compared with several other

[j] *Cx. p. pipiens* and *Cx. p. quinquefasciatus* were elevated to full species status by Sirivanakarn (Ref. 265). However, given widespread hybridization between the two taxa (e.g., Ref. 229), we feel elevation only confuses an already complex biosystematic problem.

traps.[242]

Culex tarsalis: (No common name[281]). *Culex tarsalis* is the primary enzootic and epidemic vector of SLE in agricultural areas of the western and midwestern U.S.[196,243] For a discussion of the biology of this species, see Chapter 6, WEE.

Vertebrate Host Surveillance

The bird species involved as hosts of SLE virus belong to the orders Passeriformes and Columbiformes. Populations of house sparrows, house finches, pigeons, blue jays, robins, mourning doves and cardinals, all of which are good hosts, have increased because of the expanded development of urban-suburban environments. In the west, the increase is related to the presence of irrigated farmlands. This modification of natural habitats has provided additional shelter and food. It has brought vertebrate hosts, vector mosquitoes and humans close together so virus transmission and human risk are enhanced.

In the western U.S., SLE virus activity is associated with irrigated farming regions and waterways because of the breeding habits of the principal vector, *Cx. tarsalis.* The virus regularly occurs in the valleys of California and the Great Plains states. Human cases are usually reported only sporadically in these regions, although small outbreaks have occurred recently in southern California[209] and western Colorado.[286] Although the primary SLE vector in the western states is *Cx. tarsalis*, a cycle involving birds and *Cx. pipiens* complex mosquitoes may exist in some urban locations in the west.[197,289] The house finch, mourning dove, blackbirds, house sparrow, American robin, mockingbird and pigeon are the most important avian hosts in the western transmission cycle.[288] Herons and egrets may be involved in certain locations.[176,180,179,234] A California study found domestic pigeons were inadequate as a sentinel system for SLE.[238] Pigeons developed low-titered and transient HI antibodies. Antibodies were frequently undetectable by neutralization test. In addition, pigeons were less attractive than were chickens to host-seeking *Culex* mosquitoes. Also, chickens were more sensitive sentinels for SLE virus in the Sacramento Valley of California than were either house finches or house sparrows.[233]

Throughout the central and eastern regions, human cases occur predominantly in urban environments where the *Cx. pipiens* complex mosquitoes are abundant in peridomestic environments. Birds involved with urban transmission cycles are peridomestic species such as the house sparrow and pigeon that live in close proximity to the human population and the primary urban vectors. In addition, nestlings of these species are exposed to vector mosquitoes over a long period. Their flocking behavior and sedentary nature also contribute to their importance as urban hosts.[176]

Other avian species that are involved with urban transmission are those closely associated with urban-suburban neighborhoods. These include the American robin, blue jay, cardinal, mockingbird and mourning dove. Early amplification of SLE virus transmission probably occurs within these species in areas peripheral to the urban centers. Transmission then shifts to an urban cycle involving house sparrows and pigeons by mid-summer, which provides further amplification and enhances human exposure.

Prevalences of SLE antibody in various wild bird species in urban environments are 10-50% during epizootics and 1-10% during enzootic periods.[165,176,178,180] The relative contribution of various bird species to the overall amplification of urban SLE virus depends on their local abundance and their exposure to SLE virus (Table 5-1). The specifics of an urban surveillance system using house sparrows and sentinel chickens are presented in Appendix III.

Rural transmission cycles probably occur in most regions. This could involve house sparrows and barn swallows around farms, similar to WEE transmission in the west. Other wild bird species in addition to those mentioned above (e.g., the catbird, woodthrush and bobwhite) also might be involved in woodland habitats.

In Florida, where the primary vector is *Cx. nigripalpus*, the important avian species are the pigeon, mourning dove, blue jay, cardinal and house sparrow. SLE virus transmission cycles also may involve mammals such as the raccoon and cotton rat in some areas of the state.[176]

Table 5-1. The relative contribution of species of birds to transmission of St. Louis encephalitis virus.[176]

Location & Species	Percentage of Total Avian Population	Percent Antibody Prevalence	Percentage of All Antibody-Positive Birds
Kern County, CA, 1943-1952			
House finch	25	19	55
House sparrow	20	6	14
Brewer's blackbird	25	3	9
Red-winged blackbird	9	10	10
Mourning dove	3	33	10
Tricolored blackbird	14	0	0
Other species	5	8	2
TOTAL	101	9	100
Houston, Texas 1964			
House sparrow	57	7	57
Pigeon	21	3	10
Blue jay	5	27	20
Mockingbird	3	7	3
Cardinal	1	7	2
Other species	13	4	8
TOTAL	100	8	100
Dallas, Texas 1966			
House sparrow	64	9	35
Pigeon	10	40	26
Blue jay	12	29	22
Cardinal	3	29	6
Other species	11	17	11
TOTAL	100	15	100
St. Petersburg, FL 1962-1964			
House sparrow	51	5	18
Mourning dove	20	28	37
Blue jay	12	33	26
Cardinal	4	25	6
Pigeon	2	57	6
Other species	11	9	6
TOTAL	100	26	100

Gaps in current knowledge (SLE):

Answers to the following questions could greatly improve our understanding of and ability to predict, prevent, or control epidemic transmission of EEE. We suggest that, where possible, programs should collect data that could help to provide those answers. For additional information or assistance in designing studies of this type, consult your state health department, state vector control specialist, or contact the Division of Vector-Borne Infectious Diseases, Centers for Disease Control, Fort Collins, Colorado 80522.

- What are the most reliable predictors for human risk of SLE infection?
- How can we improve the surveillance process for SLE?
- What is the overwintering mechanism of SLE virus?
- What are the human-biting habits of *Cx. p. pipiens*? Do they vary geographically or seasonally?
- What is the relationship between other potential vectors (e.g., *Cx. restuans*) and spring amplification or apparent summer transmission of SLE during the passage of cold fronts?[247]
- What is the relation between vector population age structure and the occurrence of SLE outbreaks?
- Can adult vector populations effectively be controlled? Specifically, what is the impact of control on infected vectors?
- What role does the strain of virus play in determining SLE epidemic potential?

CHAPTER 6
WESTERN EQUINE ENCEPHALOMYELITIS

Introduction

WEE virus occurs from about the Mississippi River west to the Pacific coast, (Fig. 6-1) including the prairie provinces of Canada[6] and the western states of Mexico. It occasionally produces epizootics and epidemics, but regularly causes equine and human cases.[241] Although WEE virus was previously thought to occur nationwide, it was subsequently discovered that the agent in the east was a separate virus, which was renamed Highlands J (HJ).[150] HJ virus is rarely pathogenic for horses, and is not known to be pathogenic for humans.

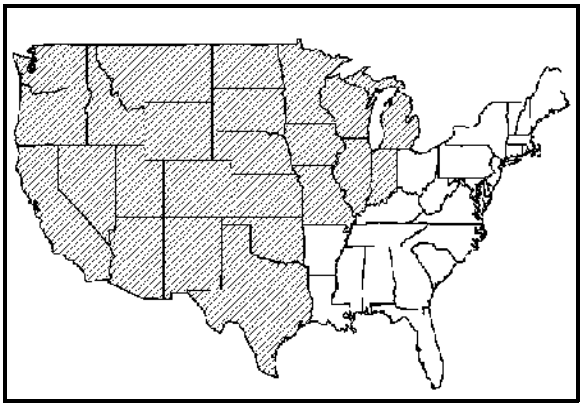

Figure 6-1. Geographic distribution of confirmed and presumptive human cases of western equine encephalomyelitis in the United States.[241]

Epidemics of WEE recur at irregular intervals or from 10 to 11 years (Fig. 6-2) For human cases reported for the period 1955 through 1992, autocorrelation analysis shows a recurrence of major activity approximately every 10 years. Reiter[247] has discussed several climatic factors that could lead to cyclic recrudescence of viruses such as WEE (Also, see below).

Meteorologic Data Monitoring

The delayed accumulation of 50 degree days above 70°F, indicating a long cool spring, has been associated with increased WEE virus transmission.[130] The date of temperature inversion in soil was shown

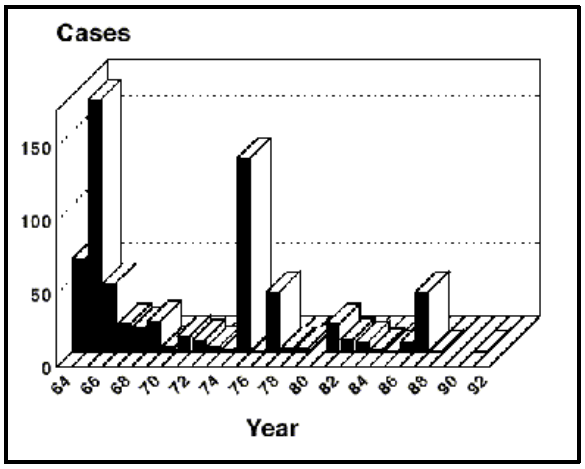

Figure 6-2. Reported cases of confirmed and presumptive human cases of western equine encephalomyelitis in the United States, 1964-1992.[l]

to correlate with the occurrence of *Cx. tarsalis*-borne WEE in humans and horses. In years of heavy snowmelt runoff or increased spring precipitation, flooding may create more larval habitats for vector species such as *Cx. tarsalis*, *Cs. inornata*, and *Aedes* spp. Prolonged cool and wet weather in spring also may increase mosquito survival. Long-lived females are more likely to become infected and transmit virus. Snowpack measurements by themselves have been variably associated with epidemic WEE transmission.

Elevated temperatures in midsummer have been associated with diminished activity of adult *Cx. tarsalis* mosquitoes; in California, this leads to reduced abundance in light trap collections in the Coachella and Imperial Valleys during August and September.[219] Infected adult females modulate their infections through prolonged hot periods, reducing transmission efficiency.[116] The relative importance of modulation and adult mortality as reducers of transmission have not been studied under field conditions. Retrospective analysis of cases in three epidemic years showed that the hottest weeks of the summer were followed by a decline in epizootic transmission. With the return of cooler temperatures, transmission resumed at a high level.[l] See Chapter 5 for an additional discussion weather and climate effects on *Cx. tarsalis*-transmitted arboviruses.

In a study comparing 2 epidemic and 2 non-epidemic years, the timing and location of WEE

[k] Tsai, T.F., P.S. Moore, and A.A. Marfin. Unpublished data.

[l] Tsai, T.F., Unpublished observations.

outbreaks in horses and humans, seroconversions in sentinel chickens, and first isolation of WEE virus from *Cx. tarsalis* could be correlated with wind trajectories from states further south.[256] It remains to be demonstrated whether there is a causal relationship between weather fronts and the appearance of WEE virus and cases.

Vector Surveillance

General information on the biology, behavior and control of WEE vectors is available in separate publications.[49,189,233,241]

Aedes melanimon: (No common name[281]). In the Sacramento Valley of California, *Ae. melanimon* is involved in a secondary transmission cycle of WEE involving jackrabbits.[145,235] This species has been reported from California, Oregon, Washington, Nevada, Utah, Idaho, Montana, Wyoming, Colorado and New Mexico, and from Alberta, Canada.

A combination of spring flooding, warming temperatures and increasing daylength stimulate eclosion of *Ae. melanimon* eggs. Larvae are commonly associated with irrigated pasture and waterfowl areas. In brackish water habitats, *Ae. melanimon* is replaced by *Ae. dorsalis.*[243] *Ae. melanimon* is multivoltine and, depending on water level fluctuations in larval habitats, can produce up to 12 or more broods per season.[243]

Peak flight activity occurs during the twilight hours in the spring and summer. However, nocturnal flight activity may increase during the fall. *Aedes melanimon* females are strong fliers. They may disperse 8 to 10 miles or more from breeding sites, particularly when aided by prevailing winds. Morning peaks in flight activity are probably associated with searches for resting sites rather than host-seeking and feeding.[243]

Aedes melanimon readily bites humans, and the species is a major pest in some areas. Leporids (hares and rabbits) serve as principal hosts. Other hosts include cattle, horses, sheep, deer and dogs. This species seldom feeds upon birds.[243] The females will bite during the day if disturbed. However, biting activity occurs primarily in the first 2 hours after sunset. There is evidence that parous females feed slightly later in the evening than nulliparous females.[243]

Daily survival has been estimated for this species in the Sacramento Valley of California.[145] Survivorship was estimated at 0.84 to 0.90 in mark-release-recapture studies, 0.82 to 0.89 in parity state studies. Another study found that about 4% and 1% of 319 specimens had completed 2 and 3 or more gonotrophic cycles, respectively.[184] Adults can be collected in large numbers in CO_2-baited CDC light traps. However, older females may be more frequently collected in New Jersey light traps.[184] This species is not readily collected from resting boxes.[184]

Culex tarsalis: (No common name[281]). *Culex tarsalis* is the primary enzootic, epizootic and epidemic vector of WEE virus in the United States.[241,243] For practical purposes WEE virus surveillance in mosquitoes can be limited to the collecting and testing of *Cx. tarsalis*. Occasional WEE virus isolates may be obtained from other mosquito species collected concurrently, or sometimes earlier in the season. The significance of such findings and their relationship to WEE virus activity are unknown.

Culex tarsalis is found from western Canada, through the United States, south to the state of Chiapas, Mexico. In Canada there are records from British Columbia, Alberta, Saskatchewan, Manitoba, and the Northwest Territories.[46] In the United States *Cx. tarsalis* is generally common west of the Mississippi River. It is usually uncommon or rare in the eastern part of the country. However, it has been collected as far east as New Jersey and Rhode Island.[65,142] The distribution of *Cx. tarsalis* shows focal clustering in the Great Plains, prairie, and other grassland areas. The vertical distribution of *Cx. tarsalis* extends from below sea level to almost 10,000 feet in California.[31]

Larval habitats of *Cx. tarsalis* are closely associated with irrigated farm and ranch lands.[186] In Kern County, California, temporary to semi-permanent earth-lined sites were the preferred larval habitat in 48% of 860 collections of this species. Only 13% of the collections came from artificially-lined containers.[243] Open, unshaded sites were preferred over shaded sites. Irrigation water, especially waste tailwater, was the most common source of larval habitats.[243]

During daylight hours the adults rest in secluded spots. A variety of natural habitats serve as resting sites. These include animal burrows, grass and shrubs, artificial shelters such as the underside of bridges. Privies, culverts, cellars, chicken houses, and other farm buildings also may serve as resting sites.

Light, temperature, and relative humidity are important variables that determine the suitability of such sites.

The seasonal abundance and duration of annual activity of *Cx. tarsalis* are influenced by latitude and temperature. Throughout much of its range the maximum adult population is reached during August or September. However, population peaks usually occur during May-June in Imperial and Coachella Valleys of southern California. In the Central Valley of California peaks have occurred in May-June, but more typically occur in July-September. Peaks have been recorded as early as July in Washington and in Alberta, Canada. Most collection records for *Cx. tarsalis* east of the Mississippi River are in late autumn. This species occurs in the Tennessee Valley from late August to late November, with a population peak in September. In west Texas *Cx. tarsalis* is abundant from June through September. Farther south in the Lower Rio Grande Valley, *Cx. tarsalis* is most abundant during November and occurs throughout the winter in appreciable numbers. Populations then begin to decline and few specimens are collected during April and May, and none from June through September. A similar situation occurs in the extreme southern valleys of California.[49,219,243]

Adults are active chiefly from dusk to dawn, with peak activity occurring within 2 hours after sunset. In a study using truck traps in Kern County, California, males were found to leave diurnal resting sites first. Males were followed by empty, blooded and gravid females, respectively.[243] Adults began returning shortly before sunrise, and entry into resting sites was in the reverse order of leaving. It is believed that most *Cx. tarsalis* females remain within 50 feet of the ground in flight,[10] although this species has been collected as high as 610 m (2,000 ft) over central Texas.[105] Dispersal occurs in all directions at low wind velocities, but mosquitoes orient into the wind as velocities increase. Winds more than 6 mph inhibit flight. *Culex tarsalis* females can travel 8 to 10 miles in 2 evenings. They may spread as far as 25 miles from breeding sites.[10]

Culex tarsalis feeds readily on humans out-of-doors during the summer months. Peak human-biting activity usually begins about 30 minutes after sunset and lasts for about l hour. Human avoidance of exposure to mosquito bites during the first couple of hours after sunset can be a practical preventive measure during the WEE transmission season. However, bites received in the early morning may have a higher probability of being infective because of increased parity among females feeding then.[243]

Precipitin test studies have shown that *Cx. tarsalis* is a general feeder with a preference for avian hosts in most areas during certain seasons of the year.[284] *Culex tarsalis* may feed almost exclusively on birds in the spring, but during the summer increasing numbers of females also feed on mammalian hosts. This shift in the feeding pattern often coincides with the appearance of WEE virus infection in humans and other vertebrates. It may be an important factor making *Cx. tarsalis* such an efficient enzootic, epizootic and epidemic vector. The reasons for the observed seasonal shift in the feeding pattern have not been fully elucidated. However, host availability, host defensive reactions mosquito density, and other seasonal variables may all play a role.[243]

Inseminated females may seek a blood meal, or in some cases may develop the first egg batch autogenously (i.e., without benefit of a blood meal).[17] The proportion of autogeny varies seasonally.[198,270] Anautogenous females will take a blood meal as early as the third day after emergence under laboratory conditions, and oviposit 4 days later. In the Central Valley of California, *Cx. tarsalis* can complete development during the summer in irrigated pastures within 9 to 10 days following irrigation.

Daily survival rates for *Cx. tarsalis* in Kern County, California have been estimated by constructing both vertical and horizontal life tables. Estimates were made at two sites from May through September over several years.[243] Seasonal mean survival rates varied from 0.63 to 0.86 per day. Estimates tended to be lower in July, possibly due to dilution by newly-emerged adults. In the Sacramento Valley of California, an emergence-independent vertical method estimated daily survival at 0.86 and 0.84 for empty and blood-fed females, respectively.[173]

Culex tarsalis females can be collected by a variety of methods. New Jersey light traps or CO_2-baited CDC light traps are effective, as are lard-can bait traps using either chickens or dry ice as bait. Walk-in or cubic-foot resting boxes can be used to collect resting females, as can aspirator collections from culverts, bridges, chicken houses, etc. In California, New Jersey light trap indices have been used to establish thresholds for virus transmission in urban and rural environments.[224] In a single California study, the Reiter gravid trap[244] was not effective in collecting *Cx. tarsalis*.[242]

Vertebrate Host Surveillance

The ecology of WEE consists of a wild bird-*Cx. tarsalis* cycle throughout the irrigated portion of western North America and along waterways in the northern plains states. Although WEE virus has been isolated from other vertebrates (rodents, jackrabbits and reptiles) and from other vectors (*Culiseta inornata* and *Aedes* spp.), only a few species of passerine birds and the principal vector, *Cx. tarsalis*, are responsible for summer amplification.[233]

The density and availability of susceptible bird species (particularly nestlings), vector density and their temporal and spatial interaction are important factors in the summer amplification of WEE. The early amplification of WEE virus transmission within the bird-mosquito cycle will increase the proportion of infected adult mosquitoes in the population. Since *Cx. tarsalis* normally shifts its host-seeking from birds to mammals in midsummer,[232,232] this higher infection ratio increases the probability of transmission of WEE to mammals when the mosquito shifts its host-feeding behavior. This increases the risk to equine and human populations.

Various measures of early viral activity have been employed to predict the occurrence of WEE cases and outbreaks. These include virus in wild avian hosts, sentinel chickens, equines or mosquito vectors, and the abundance of mosquito vectors. Monitoring WEE viral infections in birds locally involved in early amplification provides valuable information about the amount and extent of early viral transmission. This can help determine impending risk. Studies in west Texas in 1965-1969[133] demonstrated that WEE viral activity in nestling house sparrows and in *Cx. tarsalis* started by mid to late June. Activity continued in house sparrows for 8-10 weeks and in *Cx. tarsalis* for 12-13 weeks. A similar temporal pattern of virus activity was observed in North Dakota in 1975.[179] Serologic surveys in Kern County, California, found higher HI antibody prevalences against WEE virus in winter months, but WEE virus isolations were obtained from nestling birds from mid June to mid August.[233]

Surveillance programs for WEE virus vary because of differences in 1) professional orientation of the investigators, 2) ecology of vertebrate hosts and mosquito vectors, and 3) climate, physiography and agricultural practices. In Kern County, California, the birds with the highest antibody prevalence during epidemics were the house finch, house sparrow, blackbirds, orioles and mourning dove. Nestling house finches and pigeons were also valuable indicators when available.[234] Sentinel chickens were used to detect movement of WEE virus from enzootic foci to peridomestic settings before equine or human cases. A comparative study in California concluded that pigeons were less suitable than chickens as sentinels.[238]

In west Texas, infection rates in house sparrows were the best predictors of human disease.[120,133] This was true for antibody rates in free-ranging birds and for viremia in nestlings. Virus isolation rates of 5-6% in nestlings and antibody rates of 45-56% in free-ranging birds were common.[133] House sparrows were singularly useful in that area of Texas. They constituted more than two-thirds of the local avian population, were closely associated with humans and the vector mosquito, and were quite accessible for sampling.

In the northern plains states, other avian species had higher antibody prevalences and were equal in abundance and accessibility. In North Dakota house sparrows, the antibody prevalence was 13% and no virus isolations were obtained from nestlings. In contrast, there was a 46% antibody rate in the American robin. There were nine isolations of WEE virus, including seven from nestlings of four species other than house sparrows.[179] In Colorado during 1987, the antibody prevalences were 8% in house sparrows, 29% in American robins, 21% in black-capped chickadees, 15% in pigeons, 9% in red-winged blackbirds, and 7% in waterfowl.[m]

Seroconversions in sentinel chickens and equine cases have been used to monitor WEE virus activity for decades.[233] The advantages and disadvantages of using them are presented elsewhere in this publication (See Ch. I).

Gaps in current knowledge of western equine encephalitis

- What are the most reliable predictors for human risk of WEE infection?
- What predictors for WEE viral activity can be used in the Rocky Mountain and Great Plains regions?
- Are there any large-scale regional predictors for WEE viral activity?
- What is the most effective way to control vectors of WEE in an emergency (e.g., widespread flooding)?

- How can we improve surveillance for cases in humans and equines?

- Why are there few human or equine cases of WEE along the lower Colorado River in the presence of high seroconversion rates in chickens and numerous isolates from *Cx. tarsalis*?

- What is the overwintering mechanism of WEE virus?

- What is the role of wind in the dispersal of WEE vectors over regional (i.e., $\geq$ 100 km) distances?

- Are there other host-vector cycles for WEE virus (e.g., *Ae. melanimon* - jackrabbit cycle) outside California?

- Can ovarian dissection or other age-determination procedures give a more accurate estimate of the likelihood of WEE virus transmission, as with EEE in New Jersey?[64] How does autogeny impact upon parity estimates?

- Are there enzootic and epizootic/epidemic strains of WEE virus that have differing ecologies?[1]

[m] McLean, R.G., Unpublished data.

APPENDIX I
CASE DEFINITIONS AND SURVEILLANCE SYSTEMS FOR ARBOVIRAL ENCEPHALITIS

National surveillance data for human arbovirus encephalitis is collected on a monthly basis during the transmission season from April through October of each year. State and Territorial epidemiologists are encouraged to report all Probable and Confirmed cases (*see "Case definitions for arboviral encephalitis"*) using the Human Arboviral Encephalitis Surveillance Form (CDC 55.3, Figure I-1). The data are periodically summarized and reported back to State and local agencies through informal bulletins and through an annual summary of disease activity published in the MMWR. State and local public health agencies are also encouraged to immediately report outbreaks and unusual occurrences of arbovirus encephalitis directly to the Division of Vector-Borne Infectious Diseases (DVBID), NCID, CDC.

Data on arbovirus activity in wild birds and mammals, as well as in insect vectors, also are reported to the DVBID surveillance program, using CDC Forms 3.940A/B (Figure I-2). When reporting data for vectors or wild vertebrate hosts, it is helpful to have the data pooled by county (or city, if a local program). When reporting cases in equines or other domestic animals, it is very helpful to have the state case or specimen accession number. This number helps to prevent "double counting" of cases that may be reported via several systems.

Case definitions for arboviral encephalitis[52]

The following definitions are presented to assist in defining the level of certainty attached to reports of encephalitis in humans.

Possible cases of arboviral encephalitis include persons with:

a. a clinically compatible disease (febrile illness with mild neurologic symptoms, aseptic meningitis, encephalitis), AND

b. onset of illness during a period when arbovirus transmission is likely to occur.

Probable cases include persons that meet this clinical definition AND:

a. stable elevated antibody titer to an arbovirus ($\geq$ 320 by HI, $\geq$ 128 by CF, $\geq$ 256 by IFA, or $\geq$ 160 by PRNT), OR

b. specific IgM antibody in serum by EIA.

Confirmed cases of arboviral encephalitis include persons that meet this clinical definition AND:

a. fourfold or greater rise in serum antibody titer, OR

b. viral isolation from tissue, blood, or cerebrospinal fluid, OR

c. specific IgM antibody in the cerebrospinal fluid.

Existing Surveillance Programs at the State and Local Level

In 1991, state health and vector control agencies were surveyed by DVBID and the State Public Health Vector Control Conference (SPHVCC) to determine the extent and form of arboviral surveillance at the state and local level. In addition, selected large local vector control programs were included in the survey. The responses to the questionnaire are summarized in Table I-1.

It is clear that arbovirus surveillance programs vary widely in format and level of specialization. In general, large, highly developed programs tend to be located in areas with a history of arboviral encephalitis activity. However, it is probably also true that relatively more cases of arboviral encephalitis go undetected in areas that lack the capability for routine monitoring and detection of virus activity in vectors, wild vertebrate hosts, humans or domestic animals.

Table I-1. Characteristics of state arbovirus surveillance programs. Source: CDC/SPHVCC survey of state and selected local vector programs, 1991.

			Vectors		Vertebrate Hosts		Case Detection: Domest. Animals		Case Detection: Humans		Env.
State	Scope	Viruses	Count	Virus	Sentinel	Wild	Req.?	System	Req.?	System	Data
Alaska	0	...	...	...	...	...	...	...	...	...	...
Alabama	2	E,S	Y	N	Y	Y	N	P	-	P	R
Arizona	1	S,W	Y	Y	N	N	N	P	-	P	-
Arkansas	3	E,S,W	N	N	N	N	N	P	Y	P	-
California	2	S,W,O	Y	Y	Y	Y	N	P	Y	P	H,W,S
Colorado	2	S,W	Y	N	Y	N	N	P	Y	P	H,W,S
Connecticut	1	E	Y	Y	Y	Y	N	P	-	P	R,T
Delaware	1	E,S	Y	Y	Y	N	N	P	-	P	R
Florida	2	E,S,O	Y	Y	Y	Y	N	P	-	P	R,T
Georgia	3	E,S	Y	N	Y	N	N	P	-	P	R
Hawaii	0	...	...	...	...	...	...	...	...	...	...
Idaho	-	-	-	-	-	-	-	-	-	-	-
Illinois	2	E,L,S	Y	Y	Y	Y	N	P	-	P	-
Indiana	2	E,L,S,W	Y	Y	N	Y	N	P	-	P	-
Iowa	1	L,S,W	Y	Y	Y	Y	N	P	-	A	-
Kansas		S,W	N	N	N	N	Y	P	Y	P	-
Kentucky	2	E,L,S,O	N	N	N	N	N	P	Y	P	-
Louisiana	2	E,L,S	Y	Y	Y	Y	N	P	-	P	R,T
Maine	-	-	-	-	-	-	-	-	-	-	-
Maryland	1	E,S	Y	N	N	Y	N	P	-	P	R,T
Massachusetts	-	-	-	-	-	-	-	-	-	-	-
Michigan	1	E,L,S	Y	Y	Y	Y	N	P	-	P	-
Minnesota	2	L,W	Y	N	N	Y	N	P	-	P	R
Mississippi		E,S	N	N	N	N	N	P	-	P	-
Missouri	1	E,L,S,W	Y	N	N	N	N	P	-	P	-
Montana	-	-	-	-	-	-	-	-	-	-	-
Nebraska	-	-	-	-	-	-	-	-	-	-	-
Nevada	2	S,W	Y	N	Y	Y	N	P	-	P	-
New Hampshire		E	N	N	N	N	N	-	-	-	-
New Jersey	2	E	Y	Y	N	Y	N	P	-	P	R,T
New Mexico	-	-	-	-	-	-	-	-	-	-	-
New York	2	E,L,S	Y	Y	Y	Y	N	P	-	A	-
North Carolina	-	-	-	-	-	-	-	-	-	-	-
North Dakota	-	-	-	-	-	-	-	-	-	-	-
Ohio	1	E,L,S	Y	Y	Y	Y	N	-	-	P	R,T
Oklahoma	1	S,W	N	N	N	N	N	P	Y	P	H,W,S
Oregon		W	N	N	N	N	N	P	N	P	-

Pennsylvania	1	E	Y	N	Y	N	N	-	-	-	H,W,S
Rhode Island	1	E	Y	Y	N	N	N	-	-	-	-
South Carolina	-	-	-	-	-	-	-	-	-	-	-
South Dakota	1	L,S,W	N	N	N	N	N	P	-	P	-
Tennessee	3	S	Y	N	N	Y	N	-	-	-	-
Texas	2	E,S	Y	Y	N	Y	N	P	-	P	R,T
Utah	2	S,W	Y	-	Y	N	N	-	-	-	-
Vermont	0	...	...	...	...	...	...	...	...	...	...
Virginia	1	E	Y	N	N	N	N	P	-	P	-
Washington	-	-	-	-	-	-	-	-	-	-	-
West Virginia	-	-	-	-	-	-	-	-	-	-	-
Wisconsin	-	-	-	-	-	-	-	-	-	-	-
Wyoming	0	...	...	...	...	...	...	...	...	...	...

Scope:
- 0 = No program
- 1 = State level only
- 2 = State and local
- 3 = Local level only
- - = No response

Viruses:
- E = EEE
- L = Calif. Gr. (LAC, JC, CE)
- S = SLE
- W = WEE
- O = Other
- - = No response

Vectors:
- Count = Vector density from traps, etc.
- Virus = Virus isolations from vectors

Vert. Hosts:
- Sentinels = Restrained/penned animals
- Wild = Free-ranging animals

Case Detection (Domestic animals/Humans)
- Req.? = Reportable disease?
- A = Active surveillance
- P = Passive surveillance
- S = Stimulated passive surveillance
- N = No surveillance
- - = No response

Encephalitis Surveillance
Centers for Disease Control
P.O. Box 2087
Fort Collins, CO 80522-2087

Previous Form Approved
OMB No. 0920-0004

HUMAN ARBOVIRAL ENCEPHALITIS SURVEILLANCE

Current and Cumulative Data Through Week Ending Friday, ____/____/____
Month Day Year

Human Arbovirus Encephalitis NOT Previously Reported

(SLE, VEE, WEE, EEE, CE, POW)*

	Type*	Age	Sex	Conf.[1]	Pres.[2]	Town City	County	Onset Date Mo./Day	Outcome (Alive/Dead)	S1 Date	S1 Test/result	S2 Date	S2 Test/result	CSF Date	CSF Test/result
1.															
2.															
3.															
4.															
5.															
6.															

Non-human arboviral activity

Positive juvenile or hatching year bird serology? YES ____ NO ____ Type* _____

Equine encephalitis? YES ____ NO ____ Number of cases this report _____ Type* _____

Number of cumulative cases this year _____

May these data be included in the *MMWR*? YES ____ NO ____

Informant _____

State _____

Cumulative Human Arbovirus Encephalitis Cases
(Includes cases newly reported this period)

	Cumulative Cases		Cumulative Deaths	
Type*	Confirmed	Presumptive	Confirmed	Presumptive
SLE				
WEE				
EEE				
VEE				
CE				
POW				

Number of clinically suspect arbovirus encephalitis cases pending laboratory documentation _____

*SLE - St. Louis encephalitis
WEE - Western equine encephalitis
EEE - Eastern equine encephalitis
VEE - Venezuelan equine encephalitis
CE - California equine encephalitis
POW - Powassan

[1] Confirmed
[2] Presumptive

CDC 55.3 (f.4.516)
Rev.11/90

Encephalitis Surveillance
Centers for Disease Control
P.O. Box 2087
Fort Collins, CO 80522-2087

ARBOVIRUS SURVEILLANCE

Submitting Laboratory _____

Submitted by _____

Data Submission Form - Avians & Mammals

Collection interval:[1] From ___/___/___ to ___/___/___ Report date: ___/___/___

Collection Area[2]	Species	Age[3]	Test Performed	No. Tested	Virus	No. Positive[4]

(CDC 3.940A)

Data Submission Form - Mosquitoes

Collection interval:[1] From ___/___/___ to ___/___/___ Report date: ___/___/___

Collection Area[2]	Collection Method[5]	Species	Tested		No. pools positive				Test System[6]
			No.	Pools	EEE	SLE	WEE	Other	

(CDC 3.940B)

[1] Indicate interval during which birds or mosquitoes were collected.
[2] City, town, or county (not individual sites within a town).
[3] J = Juvenile, A = Adult, U = Unknown.
[4] **HI titer** $\geq$ 20.
[5] Separate data on basis of collecting method (e.g., light trap, resting station, lard can, etc.).
[6] Indicate isolation system and method(s) used for virus identification (e.g., SM/NT, Vero/IFA, etc.).

Comments: _____

_____

_____

APPENDIX II
TECHNIQUES AND EQUIPMENT FOR ADULT MOSQUITO SURVEYS

Adult mosquitoes are collected to obtain a variety of information: species composition, relative density, population age structure, arbovirus infection rates, etc. Adult surveys also can provide data on seasonal and spatial distribution of the vector(s). Depending on the type of information desired, different collection methods and equipment may be required. We must know which methods and equipment to use for a given purpose. A full discussion of the various traps and methods available is beyond the scope of these guidelines. For more detailed information, consult Service.[258]

Resting Populations

Adults of many mosquito species are inactive during the day, resting quietly in dark, cool, humid places. An index of the population density can be obtained by carefully counting the number of adults found in a resting station. These sampling sites are also a source of specimens for arbovirus tests. Sampling resting adults usually provides a representative sample of the population: collections include teneral, post-teneral unfed, blooded, and gravid females, as well as males. Population age structure also is more representative. However, different species and different gonotrophic stages may prefer different types of resting sites. Sampling resting populations is usually time consuming, especially when looking for natural resting sites. The number of specimens collected per unit of effort may be low compared to other collection methods. Mosquito resting stations are divided into two general types, natural and artificial.

"Natural" resting sites: Natural resting sites include any location not specifically constructed to serve as shelter for mosquitoes. Examples are storm sewers and culverts, bridges, houses, porches, barns, stables, chicken houses, privies, rodent burrows, tree holes and vegetation. With experience the suitability of shelters as adult mosquito resting stations is easily evaluated. Collections must be standardized for accurate comparison of results.

"Artificial" resting sites: Artificial resting stations may be constructed when suitable natural resting stations are not available. Many different types of artificial shelters have been used, including the nail keg resting station, red boxes, red cloth shelters, and privy-type shelters.[258] These shelters should be placed in shaded, humid locations near suspected breeding places or in other known congregation sites. Most species probably enter such shelters around dawn, probably in response to changes in light intensity and humidity, and ordinarily do not leave until dusk. Artificial shelter boxes, one cubic foot in size with one side open and painted red on the inside, have been used successfully for several species in the United States.[258] In studies of *Cx. tarsalis* and other species in California, walk-in red boxes have been very effective.[243]

Equipment: A variety of aspirators are available (hand-held, sweepers -- BFS, Nasci, D-Vac, etc.). In addition, specimens can be collected with a sweep net or they can be killed or immobilized by several materials (pyrethroids, chloroform, triethylamine, etc.). The de Zulueta (drop net) cage is useful for collecting specimens resting in grass or low vegetation,

Non-attractant traps

Non-attractant traps give a more representative sample of the population than attractant traps, but only sample the airborne population. A representative sample is not always desirable. For virus studies, it is better to bias collections toward collection of physiologically old females. Representative samples are highly desirable for general ecological studies. Unfortunately, these traps tend to collect few specimens. Placement is crucial. Some species may not be collected at all because they don't pass through the area where the trap is placed.

Examples of non-attractant traps include the malaise trap, the ramp trap, truck traps, sticky traps, and suction traps. For details on these traps, consult Service.[258]

Animal baits, attractants and landing/biting collections

Animal-baited and CO_2-baited traps disproportionately attract host-seeking females. This is the segment of the population of greatest interest for arbovirus surveillance. The bait species is important in trap performance. Often there is significant inter-host variability in attractiveness, which may affect trap performance. Other considerations are the duration of collection (especially human landing/biting collections), and time of day (especially important for species with a narrow host-seeking window). A final consideration is the need to decide whether to let mosquitoes feed or not (e.g., will specimens be used for

blood meal identification?). Specimens can be removed from the trap periodically with a hand aspirator.

CO_2-baited traps rely on the sublimation of dry ice (occasionally on bottled CO_2) to provide the attractant, imitating CO_2 release by the host in animal-baited traps. Another material, 1-octen-3-ol, has recently been used either alone or with CO_2 as an attractant in bait traps.[153]

Landing/biting collections, usually using humans or horses, are used to sample selected portions of the mosquito population, particularly in studies to incriminate specific vectors or in other research applications.[258] When using human bait, consideration must be given to the potential health risks involved. Particularly during epidemics, it is advisable to restrict these activities to naturally immune or immunized individuals.

Many animal-baited traps have been designed.[258] These generally are used for special studies rather than for routine surveillance. One important application for these traps is in determining the probable vector(s) of a particular virus to a given host (e.g., EEE or WEE in horses).[188,302]

Drop nets and tent traps: These traps normally are left open or are suspended above the bait (human or animal). After a set period, the openings are closed or the net lowered and the trapped mosquitoes are collected.[258] Traps can be small (e.g., for a rabbit, chicken, monkey, single human) or large (e.g., screen rooms for horses and other large animals). Large, screen rooms have been found effective in vector studies in Argentina and the U.S.[188,302]

Magoon trap: This trap is similar in principle to the tent trap, but is more substantial in design, which provides some restraint for larger bait animals.[169] Mosquitoes enter the trap but cannot escape, and they can be collected periodically. Several variations have been proposed. An interesting design uses a livestock crush or squeeze chute surrounded by a screened cage with entry baffles.[151] A modification designed for humans utilizes an inner screened enclosure that prevents the trapped mosquitoes from biting the bait/collector.[226]

Entrance/exit traps: These traps have a long history of use in malaria research.[258] A variation with application to mosquito-borne encephalitis studies is the sentinel chicken shed.[231] The trap consists of a portable chicken shed and one or more removable mosquito traps. Mosquitoes attempting to enter the shed to feed are collected in the traps and can be removed the following morning.

Small animal bait traps: Service reviews several animal-baited traps.[258] A bird-baited CDC light trap collected significantly more *Cs. melanura* and *Cs. morsitans*, but significantly fewer *Ae. vexans* when compared to a CO_2-baited CDC light trap.[97]

Lard can traps: An economical, portable mosquito trap, made from a 12-inch lard can, has been developed,[18] and is very effective in capturing *Cx. tarsalis* and *Cx. nigripalpus*. The trap is equipped with inwardly directed screen-wire funnels on each end. It utilizes about 3 pounds of dry ice (wrapped in newspaper) placed inside the can. The lard can trap also can be baited with a live chicken or other animal. An inner, double screened enclosure can be used to prevent feeding by the trapped mosquitoes.[84]

Dry ice & hand aspirator: *Ae. albopictus* adults can be collected by having the collector stand over or near a small block of dry ice. Females that are attracted by the CO_2 can be collected with a net or hand-held aspirator as they fly around the collector's legs.

DeFoliart-Morris conical trap: This is a cone trap, baited with dry ice. The attracted mosquitoes are anesthetized by the CO_2, and slide into a chamber containing dry ice where they are frozen.[77]

Duplex cone trap: Designed specifically for *Ae. albopictus*, this trap was very effective in field trials in Louisiana.[104]

Light trap with or without light: Light traps are frequently operated with dry ice as an additional attractant. For a discussion of this procedure, see "Light traps," below.

Light traps

Many mosquito species are attracted to light, making it possible to sample adult populations between dusk and dawn. Light traps probably work by disrupting the normal behavior of flying mosquitoes. Mosquito species respond differently to these traps. Some species are not attracted to light at all, and may even be repelled (e.g., *Cx. quinquefasciatus*). Light traps only sample the flying population. The catch is influenced by many factors, including light source, wavelength and intensity. Competing light sources (including moonlight, roadside lights, and"urban glow"), fan size and speed, and presence or absence of screens also affect trap performance.

Trap placement (height, location in relation

to trees and other cover, proximity to breeding sites, etc.), can have a marked effect on the species and numbers of mosquitoes collected. Some trial and error placement is frequently involved in locating good trap placement sites.

The light trap is usually suspended from a tree or post so the light is approximately 6 feet above the ground. It should be 30 feet or more from buildings, in open areas near trees and shrubs. It should not be placed near other lights, in areas subject to strong winds, or near industrial plants emit smoke or fumes. Traps should be operated on a regular schedule from one to seven nights per week, from just before dark until just after daylight.

Because differences have been noted in the reactions of different species of mosquitoes, light trap collections must be used in conjunction with other population sampling methods. Light traps are very useful in measuring densities of *Cx. tarsalis*, but less so for *Cx. p. quinquefasciatus. Culex p. pipiens* in northern areas may be collected in light traps. *Culiseta melanura* is routinely sampled with light traps in Massachusetts.

Dry ice, added as an attractant with light traps,[221] increases collections of many mosquito species including *Culex tarsalis* and *Cx. nigripalpus*. A small block of dry ice, placed in a padded shipping envelope or wrapped tightly in newspaper, is suspended a few inches above the light trap.

New Jersey light trap: The New Jersey-type light trap was developed in the early 1940's.[208] It is widely used in adult surveys because of its attraction to mosquitoes and its durability. This is a standard device used by mosquito control agencies in the United States. It can be operated manually or used with an automatic timer or photo-electric cell to start and stop the motor and light. The collection may be funneled into a killing jar. This makes the collection acceptable for relative abundance studies, but unacceptable for arbovirus studies that require live specimens. A fine-mesh collecting bag can be substituted for the killing jar when living specimens are required. Collections are gathered each morning and placed in a properly-labeled container until the mosquitoes can be sorted, identified, and counted. Live catches are processed immediately. A newly-developed antigen capture enzyme immunoassay (EIA) test can detect SLE viral antigen even in dead specimens.[287] The New Jersey-type trap depends upon a 110-volt source of electric power, which somewhat restricts its use.

CDC light trap: The CDC miniature light trap was developed for greater portability. It can be taken to remote areas that could not otherwise be sampled by a trap dependent upon electricity. It is commonly operated with four l-1/2-volt "D" cell flashlight batteries, or one 6-volt motorcycle battery, either of which provide sufficient power for one night's trapping.[277] It weighs only 1-3/4 pounds and is easily disassembled for transport. The CDC trap is fitted with a large, collapsible, nylon collecting bag (or a cardboard carton) instead of a killing jar. In this way, the catch is captured and held alive until the specimens can be frozen. The trap has a large metal or plastic canopy that shields the operating mechanism from rain. The collecting bag can be further protected in areas with heavy rain: 1) take a plastic bag large enough to fit over the mesh collecting bag, 2) cut a hole slightly larger than the diameter of the light trap body, 3) place the upside-down bag over the mesh collecting bag. Make sure the bottom of the mesh bag is unobstructed, so air can freely flow through the light trap. The CDC light trap does not compete well with other light sources and smaller catches may result during a full moon. When the CDC trap is used with CO_2 and no light, *Cx. tarsalis* can be collected without many of the other insects that are normally attracted by the light (W.C. Reeves and J.L. Hardy, personal communication, 1992). Several modifications of the CDC light trap are also commercially available.

Oviposition traps

Oviposition traps sample the gravid population. This can be an advantage for many epidemiologic studies. Since the gravid population has fed at least one time, these individuals are more likely to be infected. This reduces the work involved in processing mosquito pools for virus isolation. Minimum infection rates (MIRs) will, on average, be higher than those obtained, for example, from CDC light trap catches. Traps can be separated on the basis of whether or not they retain the ovipositing females or allow them to escape.

Ovitraps: Ovitraps only sample eggs, but the number of *Culex* rafts can be used to estimate the ovipositing (and therefore recently-fed) adult female population. Several trap designs are available for various mosquito genera and species. In general, ovitraps for *Aedes* species are small (CDC ovitrap,[99] Loor & DeFoliart[164]). Traps for *Culex* usually are larger, and usually have an attractant or infusion.[245]

Reiter gravid trap: The Reiter Gravid Trap samples female *Culex* mosquitoes as they come to oviposit.[244,246] It therefore is selective for females that have already taken at least one blood meal. If mosquitoes are being collected for virus isolation, there

is a higher probability of collecting infected mosquitoes.[248] Gravid trap counts might also have a higher correlation with disease transmission. The Harris County Mosquito Control District in Houston, Texas, has used these traps successfully in their SLE surveillance program.

APPENDIX III
VERTEBRATE SURVEILLANCE SYSTEMS

Types of Surveillance Systems

Vertebrate surveillance systems for arboviruses collect qualitative and quantitative information about the presence, distribution, intensity and temporal and spatial fluctuations in virus activity. Information can be obtained by testing specimens collected for some other purpose (passive system) or by collecting and testing specimens from vertebrates captured specifically for the surveillance program (active system). The data can be used as background information or to direct mosquito control operations to reduce the risk of human exposure. Examples of the use of vertebrate surveillance systems and useful sentinel hosts are listed below.

A. Presence and distribution of arboviruses in specific geographic area. This usually is a one time, simple, qualitative survey. It is useful to provide background information, usually detecting prevalence of antibody in free-ranging sentinels, at local, regional, or state level. The possibility of non-specific reactions should be kept in mind in this type of study.

a. Passively-collected specimens (i.e., collected for other purposes)
   1) Hunter-killed wild ungulates - statewide (EEE, SLE, WEE, JC, LAC)
   2) Trapped coyotes - predator control projects (WEE)
   3) Trapped red fox - fur trappers (LAC, EEE, JC)
   4) Rabbits or hares - trapped or hunter-killed (WEE, LAC)
   5) Waterfowl - hunter-killed or trapped (WEE, EEE, SLE)
   6) Cattle - after brucellosis testing or slaughter (WEE, JC)

b. Actively-collected specimens at selected locations
   1) Wild birds (including pigeons & house sparrows) (EEE, SLE, WEE)
   2) Chicken flocks (EEE, SLE, WEE)
   3) Raccoon (SLE, EEE, WEE)
   4) Cotton rat (or other rodents) (SLE, EEE)
   5) Eastern chipmunk and tree squirrels (LAC)
   6) Domestic dog (SLE, LAC)
   7) Equine (EEE, WEE, JC)
   8) Farm flocks (WEE, EEE, SLE)

B. Annual changes in arbovirus activity. These systems detect changes in frequency or distribution. They may be qualitative or quantitative. These generally are passive systems, and use same animal species described above. Measures include the prevalence of antibody and sometimes virus isolation. The vertebrates are generally free-ranging sentinels, although captive sentinels like chickens are sometimes used at the local-state level

C. Seasonal changes in arbovirus activity. These systems detect changes in frequency of virus or antibody. They are generally active and quantitative. The prevalence of antibody or virus is monitored in both free-ranging and captive sentinels. Such programs are usually local or regional. They are important for establishing inter-epidemic prevalence rates.

D. Within season changes in arbovirus activity. These are active and quantitative systems that monitor the prevalence of antibody or virus in tagged, free-ranging, or captive sentinels. These programs are usually local in areas with history of disease. They are important for monitoring increasing and impending risk for the human population.

E. Investigation of an epidemic (unusual occurrence). Epidemic investigations are intensive, active and quantitative studies that measure the prevalence of antibody and virus in free-ranging sentinels. These investigations are usually local or occasionally regional in scope.

Examples of Vertebrate Surveillance Programs

Two examples of well-established surveillance

programs currently in operation at the local and state level are presented below. Both are effective surveillance systems. Surveillance programs must be structured to fit the specific expertise, resources, ecology, environmental conditions, and needs of the user.

A. LOCAL SYSTEMS - Memphis, Tennessee

1. This system relies on biweekly capture of free-ranging house sparrows with mist nets at 21 sites throughout the metropolitan area from April to November. Birds are aged, sexed and tagged and a blood specimen taken before they are released at the capture site.

2. From May to October, sentinel chickens are placed at selected sites with a history of human SLE. The chickens are bled biweekly, and positive birds are re-bled for confirmation and replaced.

3. Blood samples from house sparrows and chickens are tested for SLE viral antibody within 1 day of collection by the HI or ELISA test.

4. If immature house sparrows or sentinel chickens are antibody positive, additional house sparrows are sampled within the same week at positive and adjacent sites.

5. Rapidly increasing SLE viral antibody prevalences in either sentinel system will alert the mosquito control personnel to intensify insecticide application around the positive sites or throughout the city.

6. The advantage of this system is that the surveillance and testing of sentinel birds are under the same administration as the mosquito control operations. Therefore, there is little delay in sampling and testing. More important, there is no delay in communication of results. The efforts are coordinated. Re-sampling and testing of sentinels as well as initial mosquito control can be concentrated specifically in the problem areas. There is little delay in responding to an impending risk of human disease.

7. The disadvantages of this approach include the cost of equipment and supplies, problems in establishing and maintaining quality control, and the problem of test standardization among local agencies. The cost of upgrading or changing to new technologies can be prohibitive for a local agency. Data are generally available only for a small geographic area, and nearby focal activity may not be detected. Thus, a sense of security created by treatment of identified foci of transmission could be rudely interrupted by the spread of infection from un-monitored areas.

B. STATE SYSTEMS - California State Health Department

1. Sentinel chicken flocks are set out in early spring (April-May) in pre-selected areas throughout the state. Collaboration with local mosquito control districts is emphasized.

2. Flocks of 10 chickens are bled biweekly and tested for WEE and SLE antibody at the Viral and Rickettsial Disease Laboratory (VRDL) at Berkeley.

3. Mosquitoes, mostly *Cx. tarsalis*, are collected and pooled by the mosquito control districts and tested by the VRDL by means of an in situ ELISA test.

4. Seroconversions in chickens and virus-positive mosquito pools are reported to all agencies by telephone or facsimile, as well as in the weekly VRDL reports (which also are available through the "Mosquito Net" computer bulletin board service).

5. Mosquito control operations are intensified, emphasizing adulticiding in populated areas, depending upon the findings on vector abundance, virus isolations from mosquitoes and the human population at risk. Mosquito

collections for virus isolations are intensified at the positive sites and in areas adjacent to population centers.

6. Passive reporting of suspected clinical WEE horse cases and submission of specimens for confirmation is encouraged. VRDL tests specimens for virus isolation and diagnostic rise in antibody, and reports results to the local health agency and to the veterinarian.

7. Virus surveillance activity and mosquito control operations are intensified at localities where early season (May-June) confirmed cases of WEE in horses are reported. If WEE virus is isolated from mosquito pools, local control agencies notify veterinarians and encourage them to vaccinate young and recently imported equines.

8. Advantages of this system include centralized access to advanced technology and highly trained personnel, greater ease of standardization and quality control, and state-wide comparability of results. Large geographic areas can be sampled on a routine basis. Use of the "Mosquito Net" BBS allows for rapid and widespread reporting of information to those agencies with access to the BBS.

9. Disadvantages of this system are mostly in turnaround time, particularly for seroconversion in chickens. There is a period of about 7 - 10 days after infection before antibodies are detected. Specimens are collected locally, packed, and sent to the state laboratory, which takes another 2 days. An additional 2 days are required for testing, for a turnaround time of 11 - 14 days. Since birds are bled biweekly, an additional 14 days are added for birds that have been infected but are not yet seropositive. Thus, delays of 25 - 28 days are possible between the infection of a sentinel chicken and detection of seroconversion.

Examples of Vertebrate Species Used in Surveillance Programs

Surveillance programs and epidemic investigations use many species to assess the potential for arboviral encephalitis in the United States. Table III-1 lists the most common species used.Table III-1. Common birds and mammals for arbovirus surveillance in the United States.[175]

Species	Age	Virus	Location (State)	Monitoring System
Birds				
House Sparrow	N	WEE/SLE	TX/MS	Hand capture/virus isolation
" "	I	WEE	Plains	Mist net/serology
" "	A	SLE	Midwest	" " "
Pigeons	A	SLE/WEE	Widespread	Trap/mist net/serology
Mourning dove	A	SLE	Florida	Trap/mist net/serology
House finch	A	SLE/WEE	West	Mist net/serology
Bobwhite	I	EEE/HJ	East	Sentinel cage/virus/serology
Chickens	I	WEE/SLE	Widespread	Sentinel pen/serology
"		EEE	East	" " "
Wild birds	A	SLE	Widespread	Mist net/virus/serology
" "	A	WEE	West/Plains	" " " "
" "	A	EEE	East	" " " "
Waterfowl	A	WEE/SLE	Colorado	Trap/serology
"	A	TETE	Colorado	Trap/serology
Herons/Egrets	N	WEE	Colorado	Hand capture/virus/serology
Mammals				
Cotton rat	..	SLE/VEE	Southeast	Trap/virus/serology
Gray squirrel	..	LAC	Wisconsin	Sentinel cage/virus/serology
Eastern chipmunk	..	LAC	Wisconsin	Sentinel cage/virus/serology
Rabbit	..	LAC/SSH	Wisconsin, Canada	Sentinel cage/serology
"	..	WEE/SLE	California	Shoot/serology
Red Fox	..	LAC	Wisconsin	Sentinel cage/virus/serology
Raccoon	..	SLE/EVE	Florida	Trap/virus/serology
Coyote	..	VEE/VS	Plains	Trap/serology
Dog	..	SLE/VS	Midwest	Human pet/serology
Swine	..	VS	Georgia	Trap/virus/serology
Equine	..	WEE/VEE	West	Disease case/corral/serology
"	..	EEE	East	" " " "
"	..	CV/JC	Michigan	Corral/serology
White-tailed deer	..	CE/SLE/VS	NY/Midwest	Capture/hunter-kill/serology
" " "	..	EVE/SLE	Florida	" " " "
" " "	..	SLE/VEE	Texas	" " " "
Black-tailed deer	..	CE/CV	Oregon	" " " "
" " "	..	CE/CV/NOR	California	Trap/hunter-kill/serology
Mule deer	..	CE/CV/NOR	California	" " " "
" "	..	CV/CE	California	Hunter-kill/serology
" "	..	CTF/JC/VS	Colorado	" " "
" "	..	CE/CV	Oregon	Trap/hunter-kill/serology
Pronghorn	..	WEE/JC/VS	Plains	Trap/hunter-kill/serology
Elk	..	CTF/JC/VS	Colorado	Trap/hunter-kill/serology
"	..	CE/CV	Oregon	" " " "
Big Horn Sheep	..	CE/WEE/VS	Rockies	Hunter-kill/serology

N = nestling, I = immature, A = all ages, WEE = western equine encephalitis, SLE = St. Louis encephalitis, EEE = eastern equine encephalitis, HJ = Highlands J, TETE = Tete group, VEE = Venezuelan equine encephalitis, LAC = LaCrosse, EVE = Everglades, VS = vesicular stomatitis, CV = Cache Valley, JC = Jamestown Canyon, SSH = Snowshoe hare, CE = California encephalitis, NOR = Northway, CTF = Colorado tick fever viruses; NY = New York, TX = Texas, MS = Mississippi.

INDEX

ENDNOTE LIST

[1] Agudelo-S'84
[2] Allan etal'81
[3] ACAV'85
[4] Anderson&Gallaway'87
[5] Andreadis'88
[6] Anon.'90
[7] Anon.'91
[8] Anon.'92
[9] Aziz&Hayes'87
[10] Bailey&al'65
[11] Barnard&Mulla'77
[12] Barnard&Mulla'78
[13] Barr'57
[14] Baumgartner'87
[15] Baumgartner'88
[16] Beehler etal'90
[17] Bellamy&Kardos'58
[18] Bellamy&Reeves'52
[19] Bently&al'76
[20] Bently&al'79
[21] Benyus'89
[22] Berlin'77
[23] Berry&al'86
[24] Berry&al'83
[25] Bidlingmayer'69
[26] Bidlingmayer'71
[27] Bidlingmayer'87
[28] Bidlingmayer'74
[29] Bigler'71
[30] Bigler&al'76
[31] Bohart'78
[32] Boromisa&Grayson'90
[33] Boromisa&Grimstad'86
[34] Bowen&Francy'80
[35] Breeland&al'80
[36] Burkot&Defoliart'82
[37] Calisher&al'83
[38] Calisher&al'86a
[39] Calisher&al'86b
[40] Calisher&al'86c
[41] Calisher&al'86d
[42] Calisher&al'88
[43] Callahan'87
[44] Campbell&al'91
[45] Campbell&al'89
[46] Carpenter&LaC'55
[47] CDC'76
[48] CDC'77
[49] CDC'78
[50] CDC'86
[51] CDC'86b
[52] CDC'90
[53] CDC'92
[54] Chamberlain'61
[55] Chamberlain&al'59
[56] Clark&al'83
[57] Clark&al'77
[58] Copeland&Walker'86
[59] Craig'83
[60] Crane&al'77
[61] Crane&al'83
[62] Crans'77
[63] Crans'86
[64] Crans&McCuiston'91
[65] Crans&al'79
[66] Crans&al'86
[67] Crans&al'76
[68] Crans&al'90
[69] Craven&al'88
[70] Dalrymple&al'72
[71] Darsie&Ward'81
[72] Day&Curtis'89
[73] Day&Edman'88
[74] Day&al'89
[75] DeFoliart'83
[76] DeFoliart&Lisitza'80
[77] DeFoliart&Morris'67
[78] DeFoliart&al'86
[79] Deibel&al'83
[80] Detinova'62
[81] Dixon&Brust'72
[82] Dow'71
[83] Dow&Gerrish'70
[84] Dow&al'57
[85] Dye'85
[86] Dye'92
[87] Ebsary&Crans'77a
[88] Ebsary&Crans'77b
[89] Edman'71
[90] Edman'74
[91] Edman&Taylor'68
[92] Edman&al'72a
[93] Edman&al'72b
[94] El_Hussein&al'89
[95] Eldridge'87
[96] Eldridge'90
[97] Emord&Morris'82
[98] Emord&Morris'84
[99] Fay&Eliason'66
[100] Fine'81
[101] Fish'85
[102] Fleetwood&al'78
[103] Francy&al'90
[104] Freier&Francy'91
[105] Glick&Noble'61
[106] Grady&al'78
[107] Graham&Bradley'65
[108] Grayson&al'83
[109] Grimstad'88
[110] Grimstad&al'86
[111] Gubler&al'84
[112] Gubler&al'85
[113] Gubler&al'81
[114] Haramis'84
[115] Haramis&F'83
[116] Hardy&al'83
[117] Hawley'88
[118] Hawley&al'87
[119] Hayes'75
[120] Hayes'81
[121] Hayes&al'62b
[122] Hayes&al'62a
[123] Hayes&Doane'58
[124] Hayes&Hess'64
[125] Hayes&al'67
[126] Hayes&al'73
[127] Heard&al'90
[128] Hedberg&al'85
[129] Henderson&Coleman'71
[130] Hess&al'63
[131] Holck&al'88
[132] Holden&al'73b
[133] Holden&al'73
[134] Hopkins'20
[135] Horsfall'55
[136] Horsfall&al'73
[137] Howard&al'88
[138] Howard&al'89
[139] Hutchins&al'88
[140] Irby&Apperson'88
[141] Issel'73
[142] Jakob&al'86
[143] Jakob&al'80
[144] Jennings&al'68
[145] Jensen&Washino'91
[146] Jensen&al'93
[147] Joseph&Bickley'69
[148] Kappus&al'83
[149] Karabatsos'85
[150] Karabatsos&al'63
[151] Kay&Bulfin'77
[152] Kissling&al'55
[153] Kline&al'91
[154] Knight&Stone'77
[155] Kuno&al'85
[156] LaMotte&al'67
[157] Lanciotti&al'92
[158] Landry&DeFoliart'86
[159] Landry&al'88
[160] LaSalle&Dakin'82
[161] LeDuc'79
[162] LeDuc&al'72
[163] Lomnicki'88
[164] Loor&DeFoliart'69
[165] Lord&al'74
[166] Lorenz&al'90
[167] Lounibos&'83
[168] Magnarelli'77
[169] Magoon'35
[170] Main&al'79
[171] Matsumoto'85
[172] McClure'84
[173] McHugh&Washino'86
[174] McKiel&al'66
[175] McLean'91
[176] McLean&Bowen'80
[177] McLean&al'85
[178] McLean&al'83
[179] McLean&al'89
[180] McLean&al'88
[181] McManus'88
[182] Meek&Hayes'??
[183] Meyer&al'82
[184] Meyer&al'84
[185] Milby'85
[186] Mitchell'77
[187] Mitchell'91
[188] Mitchell&al'85
[189] Mitchell&al'80
[190] Mitchell&al'73
[191] Mitchell&al'92
[192] Mitchell&al'90
[193] Mokgweetsinyana'87
[194] Mokry'84
[195] Monath'79
[196] Monath'80
[197] Monath&Tsai'87
[198] Moore'63
[199] Moore&al'90
[200] Moore&al'88
[201] Moore&al'86
[202] Morris'88
[203] Morris&al'80a
[204] Morris&al'92
[205] Morris&Srihongse'78
[206] Morris&al'80b
[207] Morris&al'76

[208] Mulhern'42
[209] Murray&al'85
[210] Muul&al'75
[211] Nasci'81
[212] Nasci'85
[213] Nasci&Edman'81a
[214] Nasci&Edman'81b
[215] Nasci&Edman'84
[216] Nayar'82
[217] Nayar&al'86
[218] Neitzel&Grimstad'91
[219] Nelson'71
[220] Nelson&al'76
[221] Newhouse&al'66
[222] Nielsen'64
[223] Olson'76
[224] Olson&al'79
[225] Parker'88
[226] Parsons'77
[227] Pickett&White'85
[228] Provost'69
[229] Pryor&Daly'91
[230] Pumpuni'89
[231] Rainey&al'62
[232] Reeves'71
[233] Reeves'90
[234] Reeves&Hammon'62
[235] Reeves&Milby'90
[236] Reeves&Milby'90b
[237] Reeves&al'90b
[238] Reisen&al'92
[239] Reisen&al'89
[240] Reisen&al'91
[241] Reisen&Monath'88
[242] Reisen&Pfuntner'87
[243] Reisen&Reeves'90
[244] Reiter'83
[245] Reiter'86
[246] Reiter'87
[247] Reiter'88
[248] Reiter&al'86
[249] Robertson&Hu'35
[250] Rosen&Gubler'74
[251] Rupp'77
[252] Schlein&Gratz'72
[253] Schreiber'88
[254] Scott&Weaver'89
[255] Scotton'79
[256] Sellers&Maarouf'88
[257] Sellers&Maarouf'90
[258] Service'76
[259] Service'85
[260] Shemanchuk'69
[261] Showers&al'89
[262] Shroyer'86
[263] Sinsko&Craig'81
[264] Sinsko'77
[265] Sirivanakarn'76
[266] Slaff&Crans'81
[267] Slaff&Crans'81a
[268] Slaff&Crans'82
[269] Smith&al'83
[270] Spadoni&al'74
[271] Spielman'64
[272] Sprance'81
[273] Sprenger&Wuith'86
[274] Stamm'58
[275] Stamm'68
[276] Stamm&al'62
[277] Sudia&Chamberlain'62
[278] Sudia&al'64
[279] Sudia&al'70
[280] Sudia&al'71
[281] Sutherland'87
[282] Taylor&al'71
[283] Taylor&al'69
[284] Tempelis'75
[285] Tsai'92
[286] Tsai&al'87
[287] Tsai&al'88
[288] Tsai&Mitchell'88
[289] Tsai&al'88b
[290] Tsai&al'89
[291] Turell&al'82
[292] Turell&al'83
[293] Walker&al'87
[294] Wallis&al'74
[295] Walsh'83
[296] Washino&al'62
[297] Watts&al'82
[298] Watts&al'73
[299] Watts&al'79
[300] Wilton'68
[301] Wilton'81
[302] Wilton&al'85
[303] Wright&DeFoliart'70
[304] Work'83
[305] Yuill'83
[306] Zavortink'72

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