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DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

Centers for Disease Control
and Prevention (CDC)
Atlanta GA 30333

December 19, 2023

Via email

This letter is our final response to your Centers for Disease Control and Prevention and Agency for Toxic Substances and Disease Registry (CDC/ATSDR) Freedom of Information Act (FOIA) request of August 18, 2023, assigned #23-01658-FOIA, for:

> *A copy of the following most recent Response Plans at CDC for each of the following conditions: Arboviral Diseases Response Plan, Hantavirus Response Plan, H1N3 Response Plan, Novel Influenza A Response Plan, Plague Response Plan, Spotted Fever Rickettsiosis Response Plan, Viral Hemorrhagic Fevers Response Plan. I believe some or all of these were produced by the Public Health Emergency Medical Countermeasures Enterprise (PHEMCE).*

We located 360 pages of responsive records and a publicly available website for Influenza response https://www.cdc.gov/flu/pandemic-resources/national-strategy/index.html. After a careful review of these pages, no information was withheld from release.

If you need any further assistance or would like to discuss any aspect of the records provided please contact either our FOIA Requester Service Center at 770-488-6399 or our FOIA Public Liaison at 770-488-6246.

Sincerely,

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23-01658-FOIA

GUIDELINES FOR ARBOVIRUS SURVEILLANCE PROGRAMS IN THE UNITED STATES

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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 (± 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 aud 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[44] 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]

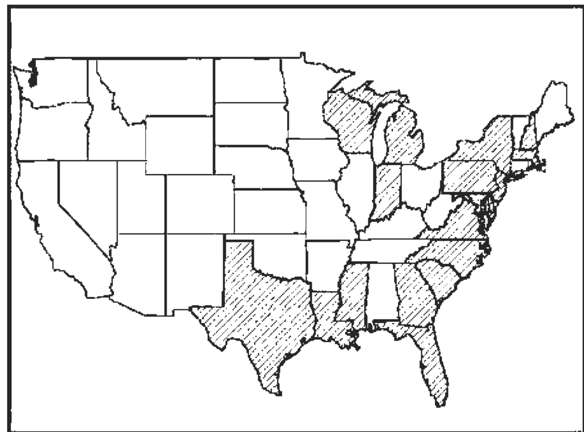

Figure 3-1. Distribution of confirmed and presumptive cases of eastern equine encephalomyelitis in the United States, 1964-1992.[e]

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.

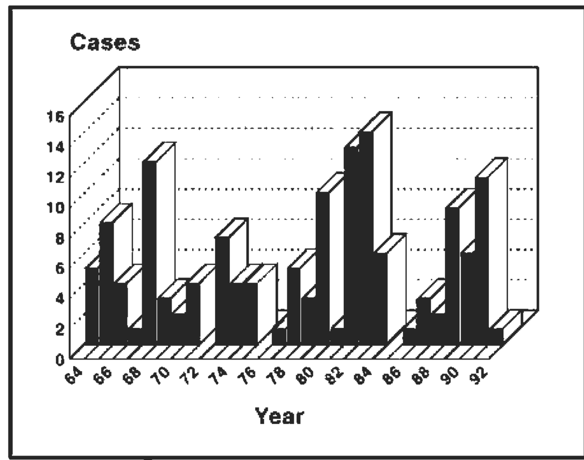

Figure 3-2. Reported cases of confirmed and presumptive human cases of eastern equine encephalomyelitis in the United States, 1964-1992.[e]

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

[e] 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*,c]). *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* prohably 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

[c] 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 fonnd throughout the Holarctic, Oriental and Pacific regions. In the New World, it is found thronghout 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 h
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.

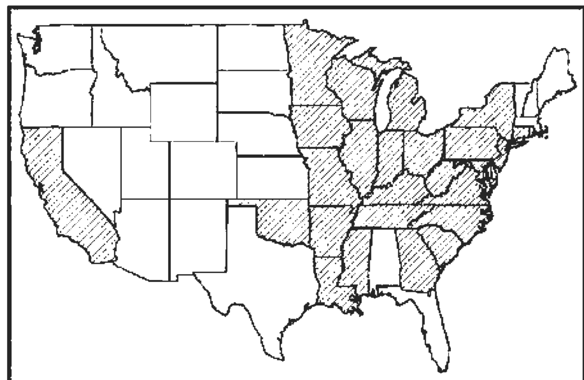

Figure 4-1. Geographic distribution of confirmed and presumptive human cases of California serogroup encephalitis (LAC, JC, CE) in the United States, 1964-1992.[g]

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.

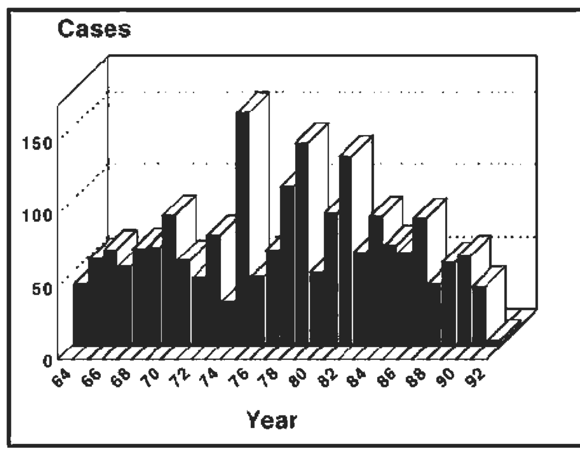

Figure 4-2. Reported confirmed and presumptive cases of encephalitis in humans due to viruses of the California serogroup (LAC, JC, CE) in the United States, 1964-1992.[g]

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 100° 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.[230] 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]

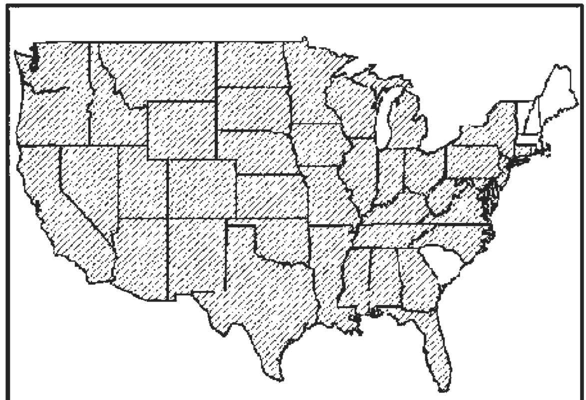

Figure 5-1. Geographic distribution of confirmed and presumptive human cases of St. Louis encephalitis in the United States, 1964-1992.[h]

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.

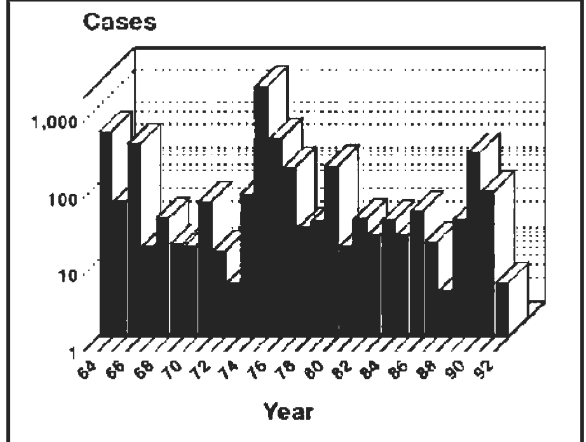

Figure 5-2. Reported cases of confirmed and presumptive human cases of St. Louis encephalitis in the United States, 1964-1992.[h]

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.

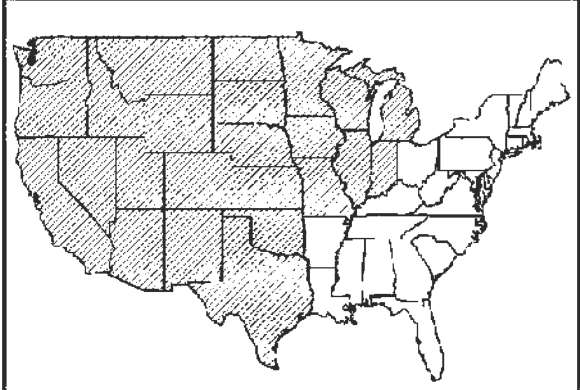

Figure 6-1. Geographic distribution of confirmed and presumptive human cases of western equine encephalomyelitis in the United States.[241]

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

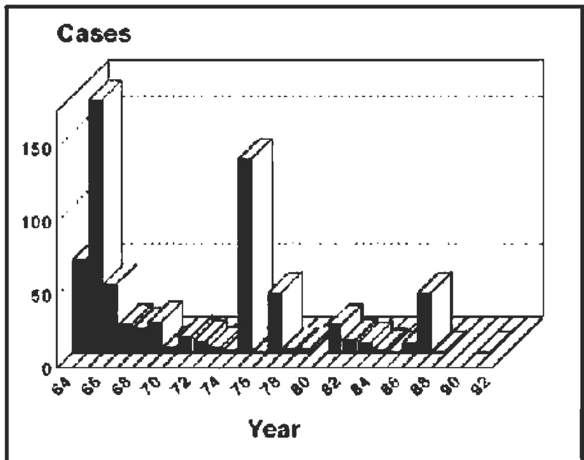

Figure 6-2. Reported cases of confirmed and presumptive human cases of western equine encephalomyelitis in the United States, 1964-1992.[l]

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. Martin. 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 1 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.[204] *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.[243] 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]

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.

							Case Detection				
			Vectors		Vertebrate Hosts		Domest. Animals		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)

Type*	Cumulative Cases: Confirmed	Cumulative Cases: Presumptive	Cumulative Deaths: Confirmed	Cumulative Deaths: 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

This report is authorized by law (Public Health Service Act 42 USC §241). Although response to the questions asked is voluntary, cooperation of the patient is necessary for the study and control of the disease. Public reporting burden for this collection of information is estimated to average 3-5 minutes per response. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to PHS Reports Clearance Officer; Rm 721-B, Humphrey Bg. 200 Independence Ave. SW; Washington, DC 20201; ATTN: PRA, Office of Management and Budget, Paper Reduction Project (0920-0004), Washington, DC 20503.

CDC 55.3 (L4 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 1-1/2-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.

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[35] Breeland&al'80
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[305] Yuill'83
[306] Zavortink'72

REFERENCES

1. Agudelo-Silva, F. and A. Spielman. 1984. Paradoxical effects of simulated larviciding on production of adult mosquitoes. Am. J. Trop. Med. Hyg. 33(6):1267-1269.

2. Allan, S.A., G.A. Surgeoner, B.V. Helson and D.H. Pengelly. 1981. Seasonal activity of *Mansonia perturbans* adults (Diptera: Culicidae) in southwestern Ontario. Canad. Ent. 113:133-139.

3. American Committee on Arthropod-borne Viruses. 1985. International catalogue of arboviruses including certain other viruses of vertebrates, 3rd ed. Karabatsos N. (ed.). American Society of Tropical Medicine and Hygiene. Address correspondence to N. Karabatsos, Centers for Disease Control, Fort Collins, CO 80522.

4. Anderson, R.A. and W.J. Gallaway. 1987. The host preferences of *Culiseta inornata* in southwestern Manitoba. J. Am. Mosq. Control Assoc. 3(2):219-221

5. Andreadis, T.G. 1988. A survey of mosquitoes breeding in used tire stockpiles in Connecticut. J. Am. Mosq. Control Assoc. 4(3):256-260.

6. Anon. 1990. A manual on guidelines for the control of arboviral encephalitides in Canada. Tech. Bull. 1990-5E. Res. Br., Agriculture Canada, Saskatoon, Saskatchewan. 216 pp.

7. Anon. 1991. The state of the state in 46 images. SpotLight (Spot Image Corp. Newsletter). March, pp. 4-5.

8. Anon. 1992. Mosquitoes in Illinois: Recommendations for prevention and control. Illinois Dept. of Public Health. 43 pp.

9. Aziz, N. and J. Hayes. 1987. Oviposition and biting patterns of *Aedes triseriatus* in the flood plains of Fort Bend County, Texas. J. Am. Mosq. Control Assoc. 3(3):397-399.

10. Bailey, S.F., D.A. Eliason and B.L. Hoffman. 1965. Flight and dispersal of the mosquito *Culex tarsalis* Coquillett in the Sacramento Valley of California. Hilgardia 37(3):73-113.

11. Barnard, D.R. and M.S. Mulla. 1977. Diel periodicity of blood feeding in the mosquito *Culiseta inornata* in the Coachella Valley of southern California. Mosq. News 37(4):669-673.

12. Barnard, D.R. and M.S. Mulla. 1978. Seasonal variation of lipid content in the mosquito *Culiseta inornata*. Ann. Ent. Soc. Am. 71(4):637-639.

13. Barr, A.R. 1957. The distribution of *Culex pipiens pipiens* and *Culex pipiens quinquefasciatus* in North America. Am. J. Trop. Med. Hyg. 6:153-165.

14. Baumgartner, D.L. 1987. Importance of construction sites as foci for urban *Culex* in northern Illinois. J. Am. Mosq. Control Assoc. 3(1):26-34.

15. Baumgartner, D.L. 1988. Suburban accumulations of discarded tires in northeastern Illinois and their associated mosquitoes. J. Am. Mosq. Control Assoc. 4(4):500-508.

16. Beehler, J.W. and G.R. DeFoliart. 1990. A field evaluation of two suggested *Aedes triseriatus* oviposition attractants. J. Am. Mosq. Control Assoc. 6(4):720-722.

17. Bellamy, R.H. and E.H. Kardos. 1958. A strain of *Culex tarsalis* reproducing without a blood meal. Mosq. News 18:132-134.

18. Bellamy, R.E. and W.C. Reeves. 1952. A portable mosquito bait-trap. Mosq. News 12(4):256-258.

19. Bently, M.D., I.N. McDaniel, H.-P. Lee, B. Stiehl and M. Yatagai. 1976. Studies of *Aedes triseriatus* oviposition attractants produced by larvae of *Aedes triseriatus* and *Aedes atropalpus* (Diptera: Culicidae). J. Med. Ent. 13(1):112-115.

20. Bently, M.D., E.N. McDaniel, M. Yatagai, H.-P. Lee and R. Maynard. 1979. p-Cresol: an oviposition attractant of *Aedes triseriatus*. Env. Ent. 8(2):206-209.

21. Benyus, J.M. 1989. A Field Guide to Wildlife Habitats of the Eastern United States. Simon & Schuster, Inc., New York.

22. Berlin, J.A. 1977. The occurrence of *Aedes sollicitans* in western New York. Mosq. News 37(3):521-522.

23. Berry, R.L., M.A. Parsons, B.J. Lalonde-Weigert, J. Lebio, H. Stegmiller and G.T. Bear. 1986. *Aedes canadensis*, a vector of La Crosse virus (California serogroup) in Ohio. J. Am. Mosq. Control Assoc. 2(1):73-78.

24. Berry, R.L., M.A. Parsons, R.A. Restifo, E.D. Peterson, S.W. Gordon, M.R. Reed, C.H. Calisher, G.T. Bear and T.J. Halpin. 1983. California serogroup virus infections in Ohio: An 18-year retrospective summary. pp. 215-233. In: C.H. Calisher and W.H. Thompson (eds.) California Serogroup Viruses. Alan R. Liss, Inc., New York.

25. Bidlingmayer, W.L. 1969. The use of logarithms in analyzing trap collections. Mosq. News 29(4):635-640.

26. Bidlingmayer, W.L. 1971. Mosquito flight paths in relation to the environment. 1. Illumination levels, orientation, and resting areas. Ann. Ent. Soc. Am. 64(5):1121-1131.

27. Bidlingmayer, W.L. and D.G. Evans. 1987. The distribution of female mosquitoes about a flight barrier. J. Am. Mosq. Control Assoc. 3(3):369-377.

28. Bidlingmayer, W.L., B.P. Franklin, A.M. Jennings and E.F. Cody. 1974. Mosquito flight paths in relation to the environment. Influence of blood meals, ovarian stage and parity. Ann. Ent. Soc. Am. 67(6):919-927.

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

30. Bigler, W.J., E.B. Lassing, E.E. Buff, E.C. Prather, E.C. Beck and G.L. Hoff. 1976. Endemic eastern equine encephalomyelitis in Florida: a twenty-year analysis, 1955-1974. Am. J. Trop. Med. Hyg. 25:884-890.

31. Bohart, R.M. and R.K. Washino. 1978. Mosquitoes of California (3d ed.). Univ. of Calif., Div. Agric. Sci., Pub. No. 4084.153 pp.

32. Boromisa, R.D. and M.A. Grayson. 1990. Incrimination of *Aedes provocans* as a vector of Jamestown Canyon virus in an enzootic focus of northeastern New York. J. Am. Mosq. Control Assoc. 6(3):504-509.

33. Boromisa, R.D. and P.R. Grimstad. 1986. Virus-vector-host relationships of *Aedes stimulans* and Jamestown Canyon virus in a northern Indiana enzootic focus. Am. J. Trop. Med. Hyg. 35(6):1285-1295.

34. Bowen, G.S. and D.B. Francy. 1980. Surveillance. pp. 473-499. In: T.P. Monath (ed.) St. Louis Encephalitis. Am. Pub. Hlth. Assoc., Washington, DC.

35. Breeland, S.G., R.T. Taylor & C.J. Mitchell. 1980. Control of mosquito vectors of St. Louis encephalitis virus. (Ch. 13). In: T.P. Monath (ed). St. Louis Encephalitis. Am. Pub. Hlth. Assoc., Washington, DC.

36. Burkot, T.R. and G.R. DeFoliart. 1982. Bloodmeal sources of *Aedes triseriatus* and *Aedes vexans* in a southern Wisconsin forest endemic for LaCrosse encephalitis virus. Am. J. Trop. Med. Hyg. 31:376-381.

37. Calisher, C.H., J.K. Emerson, D.J. Muth, J.S. Lazuick and T.P. Monath. 1983. Serodiagnosis of western equine encephalitis virus infection in equines: Relationship of antibody titer and test employed to observed onset of clinical signs. J. Am. Vet. Med. Assoc. 183:438-440.

38. Calisher, C.H., V.P. Berardi, D.J. Muth and E.E. Buff. 1986. Specificity of immunoglobulin M and G antibody responses in humans infected with eastern equine encephalitis and western equine encephalitis viruses: Applications to rapid serodiagnosis. J. Clin. Micro. 23:369-372.

39. Calisher, C.H., H.N. Fremount, W.L. Vesely, A.O. El-Kafrawi, and M.I. Al-D. Mahmud. 1986. Rapid detection of immunoglobulin M antibody in sentinel chickens used for arbovirus surveillance. J. Clin. Micro. 24:770-774.

40. Calisher, C.H., M.I.Al-D. Mahmud, A.O. El-Kafrawi, J.K. Emerson and D.J. Muth. A method for the rapid and specific serodiagnosis of western equine encephalitis virus infections in equines. Am. J. Vet. Res. 47:1296-1299.

41. Calisher, C.H., C.I. Pretzman, D.J. Muth, M.A. Parsons and E.D. Peterson. 1986. Serodiagnosis of La Crosse virus infections in humans by detection of immunoglobulin M class antibodies. J. Clin. Micro. 23:667-671.

42. Calisher, C.H., R.E. Shope and T.E. Walton. 1988. Application of cell cultures to diagnosis of arbovirus infections of livestock and wildlife. J. Tissue Cult. Meth. 11:157-163.

43. Callahan, J.L. and C.D. Morris. 1987. Survey of 13 Polk County, Florida lakes for mosquito (Diptera: Culicidae) and midge (Diptera: Chironomidae) production. Florida Ent. 70(4):471-478.

44. Campbell, G.L., B.F. Eldridge, W.C. Reeves and J.L. Hardy. Isolation of Jamestown Canyon virus from boreal *Aedes* mosquitoes from the Sierra Nevada of California. Am. J. Trop. Med. Hyg. 44(3):244-249.

45. Campbell, G.L., B.F. Eldridge, J.L. Hardy, W.C. Reeves, D.A. Jessup and S.B. Presser. 1989. Prevalence of neutralizing antibodies against California and Bunyamwera serogroup viruses in deer from mountainous areas of California. Am. J. Trop. Med. Hyg. 40(4):428-437.

46. Carpenter, S.J. and W.J. LaCasse. 1955. Mosquitoes of North America. Univ. Calif. Press, Berkeley & Los Angeles. 153 pp.

47. CDC. 1976. Control of St. Louis encephalitis. Vector Topics No. 1. U.S. Dept. of Health aud Human Svcs., Atlanta, GA. 35 pp.

48. CDC. 1977. Mosquitoes of Public Health Importance and Their Control. HHS Publ. No. (CDC) 82-8140. U.S. Dept. of Health & Human Svcs., Atlanta, GA. 55 pp.

49. CDC. 1978. Control of western equine encephalitis. Vector Topics No. 3. U.S. Dept. of Health and Human Svcs., Atlanta, GA. 35 pp.

50. CDC. 1986. Arboviral infections of the central nervous system--United States, 1985. Morb. Mort. Wkly. Rpt. 35(21):341-350.

51. CDC. 1986. *Aedes albopictus* introduction - Texas. Morb. Mort. Wkly. Rpt. 35(9):141-142.

52. CDC. 1990. Case definitions for public health surveillance. Morb.Mort. Wkly Rpt. 39(RR-13):11-12.

53. CDC. 1992. Eastern equine encephalitis virus associated with *Aedes albopictus* - Florida, 1991. Morb. Mort. Wkly. Rpt. 41(7):115,121.

54. Chamberlain, R.W. and W.D. Sudia. 1961. Mechanism of transmission of viruses by mosquitoes. Annu. Rev. Ent. 6:371-390.

55. Chamberlain, R.W., W.D. Sudia and J.D. Gillett. 1959. St. Louis encephalitis virus in mosquitoes. Am. J. Hyg. 70:221-236.

56. Clark, G.G., H.L. Pretula, W.H. Rohrer, R.N. Harroff & T. Jakubowski. 1982. Persistence of La Crosse virus (California encephalitis serogroup) in north-central Illinois. Am. J. Trop. Med. Hyg. 32(1):175-184.

57. Clark, G.G., H.L. Pretula, T. Jakubowski and M.A. Hurd. 1977. Arbovirus surveillance in Illinois in 1976. Mosq. News 37(3):389-395.

58. Copeland, R.S. and E.D. Walker. 1986. Sewage-associated breeding of *Aedes sollicitans* and *Aedes dorsalis* in southwestern Michigan. J. Am. Mosq. Control Assoc. 2(1):91.

59. Craig, G.B. 1983. Biology of *Aedes triseriatus:* Some factors affecting control. pp. 320-341. In: Calisher, C.H. (ed.). California Serogroup Viruses. Alan R. Liss, New York.

60. Crane, G.T., R.E. Elbel and C.H. Calisher. 1977. Transovarial transmission of California encephalitis virus in the mosquito *Aedes dorsalis* at Blue Lake, Utah. Mosq. News 37(3):479-482.

61. Crane, G.T., R.E. Elbel, D.B. Francy and C.H. Calisher. 1983. Arboviruses from western Utah, USA, 1967-1976. J. Med. Ent. 20(3):294-300.

62. Crans, W.J. 1977. The status of *Aedes sollicitans* as an epidemic vector of eastern equine encephalitis in New Jersey. Mosq. News. 37(1):85-89.

63. Crans, W.J. 1986. Failure of chickens to act as sentinels during an epizootic of eastern equine encephalitis in southern New Jersey, USA. J. Med. Ent. 23(6):626-629.

64. Crans, W.J. and L.J. McCuiston (In Press). New Jersey's approach to encephalitis prevention. Bull. Soc. Vector Ecol.

65. Crans, W.J., F. Lesser and T. Candeletti. 1979. Recent distribution records of *Culex tarsalis* in New Jersey. Mosq. News 39(2):244-247.

66. Crans, W.J., J. McNelly, T.L. Schulze and A. Main. 1986. Isolation of eastern equine encephalitis virus from *Aedes sollicitans* during an epizootic in southern New Jersey. J. Am. Mosq. Control Assoc. 2(1):68-72.

67. Crans, W.J., J.D. Downing and M.E. Slaff. 1976. Behavioral changes in the salt marsh mosquito, *Aedes sollicitans*, as a result of increased physiological age. Mosq. News 36(4):437-445.

68. Crans, W.J., L.J. McQuiston and D.A. Sprenger. 1990. The blood-feeding habits of *Aedes sollicitans* (Walker) in relation to eastern equine encephalitis virus in coastal areas of New Jersey. I. Host selection in nature determined by precipitin tests on wild-caught specimens. Bull. Soc. Vector Ecol. 15(2):144-148.

69. Craven, R.B., D.A. Eliason, D.B. Francy, P. Reiter, E.G. Campos, W.L. Jakob, G.C. Smith, C.J. Bozzi, C.G. Moore, G.O. Maupin and T.P. Monath. 1988. Importation of *Aedes albopictus* and other exotic mosquito species into the United States in used tires from Asia. J. Am. Mosq. Control Assoc. 4(2):138-142.

70. Dalrymple, J.M., O.P. Young, B.F. Eldridge and P.K. Russell. 1972. Ecology of arboviruses in a Maryland freshwater swamp. III. Vertebrate hosts. Am. J. Epidemiol. 96:129-140.

71. Darsie, R.F. and R.A. Ward. 1981. Identification and geographical distribution of the mosquitoes of North America north of Mexico. Mosq. Syst. Supplement 1:1-313.

72. Day, J.F. and G.A. Curtis. 1989. Influence of rainfall on *Culex nigripalpus* (Diptera: Culicidae) blood-feeding behavior in Indian River County, Florida. Ann. Ent. Soc. Am. 82(1):32-37.

73. Day, J.F. and J.D. Edman. 1988. Host location, blood-feeding, and oviposition behavior of *Culex nigripalpus* (Diptera: Culicidae): Their influence on St. Louis encephalitis virus transmission in southern Florida. In: T.W. Scott & J. Grumstrup-Scott (eds.) Proceedings of a Symposium: The Role of Vector-Host Interactions in Disease Transmission. Misc. Pubs. Ent. Soc. Am. 68:1-8.

74. Day, J.F., G.A. Curtis and J.D. Edman. 1989. Rainfall-directed oviposition behavior of *Culex nigripalpus* (Diptera: Culicidae) and its influence on St. Louis encephalitis virus transmission in Indian River County, Florida. J. Med. Ent. 27(1):43-50.

75. DeFoliart, G.R. 1983. *Aedes triseriatus:* Vector biology in relationship to the persistence of LaCrosse virus in Endemic foci. pp. 89-104. In: Calisher, C.H. (ed.). California Serogroup Viruses. Alan R. Liss, New York.

76. DeFoliart, G.R. and M.A. Lisitza. 1980. Activity by *Aedes triseriatus* in open terrain. Mosq. News 40(4):650-652.

77. DeFoliart, G.R. and C.D. Morris. 1967. A dry ice-baited trap for the collection and field storage of hematophagous Diptera. J. Med. Ent. 4(3):360-362.

78. DeFoliart, G.R., D.M. Watts and P.R. Grimstad. 1986. Changing patterns in mosquito-borne arboviruses. J. Am. Mosq. Control Assoc. 2(4):437-455.

79. Deibel, R., S. Srihongse, M.A. Grayson, P.R. Grimstad, M.S. Mahdy, H. Artsob and C.H. Calisher. 1983. Jamestown Canyon virus: The etiologic agent of an emerging human disease? pp 257-267. In: C.H. Calisher and W.H. Thompson (eds.) California Serogroup Viruses. Alan R. Liss, Inc., New York.

80. Detinova, T.S. 1962. Age-grading methods in Diptera of medical importance. Wld. Hlth. Org. Monogr. Ser. 47:1-216.

81. Dixon, R.D. and R.A. Brust. 1972. Mosquitoes of Manitoba. III. Ecology of larvae in the Winnipeg area. Canad. Ent. 104(7):961-968.

82. Dow, R.P. 1971. The dispersal of *Culex nigripalpus* marked with high concentrations of radiophosphorus. J. Med. Ent. 8:353-363.

83. Dow, R.P. and G.M. Gerrish. 1970. Day-to-day change in relative humidity and the activity of *Culex nigripalpus* (Diptera: Culicidae). Ann. Ent. Soc. Am. 63(4):995-999.

84. Dow, R.P., W.C. Reeves and R.E. Bellamy. 1957. Field tests of avian host preference of *Culex tarsalis* Coq. Am. J. Trop. Med. Hyg. 6(2):294-303.

85. Dye, C. and G. Hasibeder. 1985. Patterns of mosquito-host contact and disease population dynamics. In: L.P. Lounibos et al. Ecology of Mosquitoes: Proceedings of a Workshop. Univ. Florida Press. pp. 165-272.

86. Dye, C. The analysis of parasite transmission by bloodsucking insects. Annu. Rev. Entomol. 37:1-19.

87. Ebsary, B.A. and W.J. Crans. 1977. The biting activity of *Aedes sollicitans* in New Jersey. Mosq. News 37(4):721-724.

88. Ebsary, B.A. and W.J. Crans. 1977. The physiological age structure of an *Aedes sollicitans* population in New Jersey. Mosq. News 37(4):647-653.

89. Edman, J.D. 1971. Host-feeding patterns of Florida mosquitoes. I. *Aedes, Anopheles, Coquillettidia, Mansonia* and *Psorophora*. J. Med. Ent. 8(6):687-695.

90. Edman, J.D. 1974. Host-feeding patterns of Florida mosquitoes. III. *Culex (Culex)* and *Culex (Neoculex)*. J. Med. Ent. 11(1):95-104.

91. Edman, J.D. and D.J. Taylor. 1968. *Culex nigripalpus:* Seasonal shift in the bird-mammal feeding ratio in a mosquito vector of human encephalitis. Science 161:67-68.

92. Edman, J.D., L.A. Webber and H.W. Kale. 1972. Effect of mosquito density on the interrelationship of host behavior and mosquito feeding success. Am. J. Trop. Med. Hyg. 21(4):487-491.

93. Edman, J.D., L.A. Webber and H.W. Kale. 1972. Host-feeding patterns of Florida mosquitoes. II. *Culiseta*. J. Med. Ent. 9(5):429-434.

94. El Hussein, A., C.H. Calisher, F.R. Holbrook, R.J. Schoepp and B.J. Beaty. 1989. Detection of bluetongue virus antigens in *Culicoides variipennis* by enzyme immunoassay. J. Clin. Micro. 27:1320-1323.

95. Eldridge, B.F. 1987. Strategies for surveillance, prevention, and control of arbovirus diseases in western North America. Am. J. Trop. Med. Hyg. 37(3S):77s-86s.

96. Eldridge, B.F. 1990. Daily survivorship of adult *Aedes communis* in a high mountain environment in California. J. Am. Mosq. Control Assoc. 6(4):662-666.

97. Emord, D.E. and C.D. Morris. 1982. A host-baited CDC trap. Mosq. News 42(2):220-224.

98. Emord, D.E. and C.D. Morris. 1984. Epizootiology of eastern equine encephalomyelitis virus in upstate New York, USA. VI. Antibody prevalence in wild birds during an interepizootic period. J. Med. Ent. 21:395-404.

99. Fay, R.W. and D.A. Eliason. 1966. A preferred oviposition site as a surveillance method for *Aedes aegypti*. Mosq. News 26:531-535.

100. Fine, P.E.M. 1981. Epidemiological principles of vector-mediated transmission. pp. 77-91. In: McKelvey, J.J., B.F. Eldridge and K. Maramorosch (eds). Vectors of Disease Agents. Praeger Publishers, New York, NY.

101. Fish, D. 1985. An analysis of adult size variation within natural mosquito populations. pp. 419-429. In: L.P. Lounibos, J.R. Rey & J.H. Frank (eds.) Ecology of Mosquitoes: Proceedings of a Workshop. Florida Medical Entomology Laboratory, Vero Beach, FL.

102. Fleetwood, S.C., C.D. Steelman and P.E. Schilling. 1978. The association of certain permanent and floodwater mosquitoes with selected plant species in Louisiana coastal marshes. Env. Ent. 7(3):300-304.

103. Francy, D.B., N. Karabatsos, D.M. Wesson, C.G. Moore, J.S. Lazuick, M.L. Niebylski, T.F. Tsai and G.B. Craig. 1990. A new arbovirus from *Aedes albopictus*, an Asian mosquito established in the United States. Science 250:1738-1740.

104. Freier, J.E. and D.B. Francy. 1991. A duplex cone trap for the collection of adult *Aedes albopictus*. J. Am. Mosq. Control Assoc. 7(1):73-79.

105. Glick, P.A. and L.W. Noble. 1961. Airborne movement of the pink bollworm and other arthropods. USDA/ARS, Tech. Bull. 1255. (Cited in 10).

106. Grady, G.F., H.K. Maxfield, S.W. Hildreth, R.J. Timperi, Jr., R.F. Gilfillan, B.J. Rosenau, D.B. Francy, C.H. Calisher, L.C. Marcus and M.A. Madoff. 1978. Eastern equine encephalitis in Massachusetts, 1957-1976: A prospective study centered upon analyses of mosquitoes. Am. J. Epidemiol. 107:170-178.

107. Graham, J.E. and I.E. Bradley. 1965. *Culiseta inornata* (Williston) and temperature in Utah. Mosq. News 25(2):107-111.

108. Grayson, M.A., S. Srihongse, R. Deibel and C.H. Calisher. 1983. California serogroup viruses in New York state: A retrospective analysis of subtype distribution patterns and their epidemiologic significance, 1965-1981. pp. 257-267. In: C.H. Calisher (ed.) California Serogroup Viruses. Alan R. Liss, New York.

109. Grimstad, P.R. 1988. California group virus disease. pp. 99-136. In: T.P. Monath (ed.) The Arboviruses: Epidemiology and Ecology. Vol. II. CRC Press, Inc., Boca Raton, FL.

110. Grimstad, P.R., C.H. Calisher, R.N. Harroff and B.B. Wentworth. 1986. Jamestown Canyon virus (California serogroup) is the etiologic agent of widespread infection in Michigan humans. Am. J. Trop. Med. Hyg. 35(2):376-386.

111. Gubler, D.J., G. Kuno, G.E. Sather, M. Velez and A. Oliver. 1984. Mosquito cell cultures and specific monoclonal antibodies in surveillance for dengue viruses. Am. J. Trop. Med. Hyg. 33(1):158-165.

112. Gubler, D.J., G. Kuno, G.E. Sather and S.H. Waterman. 1985. A case of natural concurrent human infection with two dengue viruses. Am. J. Trop. Med. Hyg. 34(1):170-173.

113. Gubler, D.J., W. Suharyono, R. Tan, M. Abidin and A. Sie. 1981. Viraemia in patients with naturally acquired dengue infection. Bull. Wld. Hlth. Org. 59(4):623-630.

114. Haramis, L.D. 1984. *Aedes triseriatus:* A comparison of density in tree holes vs. discarded tires. Mosq. News 44(4):485-489.

115. Haramis, L.D. and W.A. Foster. 1983. Survival and population density of *Aedes triseriatus* (Diptera: Culicidae) in a woodlot in central Ohio, USA. J. Med. Ent. 20(4):291-398.

116. Hardy, J.L., E.J. Houk, L.D. Kramer and W.C. Reeves. 1983. Intrinsic factors affecting vector competence of mosquitoes for arboviruses. Annu. Rev. Ent. 28:229-262.

117. Hawley, W.A. 1988. The biology of *Aedes albopictus*. J. Am. Mosq. Control Assoc. 4(Suppl. 1):1-40.

118. Hawley, W.A., P. Reiter, R.S. Copeland, C.B. Pumpuni and G.B. Craig. 1987. *Aedes albopictus* in North America: Probable introduction in tires from northern Asia. Science 236:1114-1116.

119. Hayes, J. 1975. Seasonal changes in population structure of *Culex quinquefasciatus* Say (Diptera: Culicidae): Study of an isolated population. J. Med. Ent. 12(2):167-178.

120. Hayes, R.O. 1981. Eastern and western equine encephalitis. pp. 29-57. In: J.H. Steele (ed.), CRC Handbook Series in Zoonoses, Sect. B, Viral Zoonoses, Vol 1.

121. Hayes, R.O., L.D. Beadle, A.D. Hess, O. Sussman and M.J. Bones. 1962. Entomological aspects of the 1959 outbreak of eastern encephalitis in New Jersey. Am. J. Trop. Med. Hyg. 11:115-121.

122. Hayes, R.O., J.B. Daniels, K.S. Anderson, M.A. Parsons, H.K. Maxfield and L.C. LaMotte. 1962. Detection of eastern equine encephalitis virus and antibody in wild and domestic birds in Massachusetts. Am. J. Hyg. 75:183-189.

123. Hayes, R.O. and O.W. Doane, Jr. 1958. Primary record of *Culiseta melanura* biting man in nature. Mosq. News 18(3):216-217.

124. Hayes, R.O. and A.D. Hess. 1964. Climatological conditions associated with outbreaks of eastern encephalitis. Am. J. Trop. Med. Hyg. 13:851-858.

125. Hayes, R.O., L.C. LaMotte and P. Holden. 1967. Ecology of arboviruses in Hale County, Texas, during 1965. Am. J. Trop. Med. Hyg. 16:675-687.

126. Hayes, R.O., C.H. Tempelis, A.D. Hess and W.C. Reeves. 1973. Mosquito host preference studies in Hale County, Texas. Am. J. Trop. Med. Hyg. 22(2):270-277.

127. Heard, P.B., M.-B. Zhang and P.R. Grimstad. 1990. Isolation of jamestown Canyon virus (California serogroup) from *Aedes provocans* mosquitoes in an enzootic focus in Michigan. J. Am. Mosq. Control Assoc. 6(3):461-468.

128. Hedberg, C.W., J.W. Washburn and R.D. Sjogren. 1985. The association of artificial containers and LaCrosse encephalitis cases in Minnesota, 1979. J. Am. Mosq. Control Assoc. 1(1):89-90.

129. Henderson, B.E. and P.H. Coleman. 1971. The growing importance of California arboviruses in the etiology of human disease. Prog. Med. Virol. 13:404-461.

130. Hess, A.D., C.E. Cherubin and L. LaMotte. 1963. Relation of temperature to activity of western and St. Louis encephalitis viruses. Am. J. Trop. Med. Hyg. 12:657-667.

131. Holck, A.R., C.L. Meek and J.C. Holck. 1988. Attractant enhanced ovitraps for the surveillance of container breeding mosquitoes. J. Am. Mosq. Control Assoc. 4:97-98.

132. Holden, P., D.B. Francy, C.J. Mitchell, R.O. Hayes, J.S. Lazuick and T.B. Hughes. 1973. House sparrows, *Passer domesticus*, as hosts of arboviruses in Hale County, Texas. II. Laboratory studies with western equine encephalitis virus. Am. J. Trop. Med. Hyg. 22:254-262.

133. Holden, P., R.O. Hayes, C.J. Mitchell, D.B. Francy, J.S. Lazuick, and T.B. Hughes. 1973. House sparrows, *Passer domesticus* (L.), as hosts of arboviruses in Hale County, Texas. I. Field studies, 1965-1969. Am. J. Trop. Med. Hyg. 22: 244-253.

134. Hopkins, A.D. 1920. The bioclimatic law. J. Wash. Acad. Sci. 10:34-40.

135. Horsfall, W.R. 1955. Mosquitoes, Their Bionomics and Relation to Disease. Ronald Press, New York. 723 pp.

136. Horsfall, W.R., H.W. Fowler, L.J. Moretti and J.R. Larsen. 1973. Bionomics and Embryology of the Inland Floodwater Mosquito, *Aedes vexans*. Univ. Illinois Press, Urbana, IL. 211 pp.

137. Howard, J.J., C.D. Morris, D.E. Emord and M.A. Grayson. 1988. Epizootiology of eastern equine encephalitis virus in upstate New York, USA. VII. Virus surveillance 1978-85, description of 1983 outbreak, and series conclusions. J. Med. Ent. 25(6):501-514.

138. Howard, J.J., D.J. White and S.L. Muller. 1989. Mark-recapture stndies on the *Culiseta* (Diptera: Culicidae) vectors of eastern equine encephalitis virus. J. Med. Ent. 26(3):190-199.

139. Hutchins, S.H., R.B. Smelser and L.P. Pedigo. 1988. Insect migration: Atmospheric modeling and industrial application of an ecological phenomenon. Bull. Ent. Soc. Am. Spring, 1988.

140. Irby, W.S. and C.S. Apperson. 1988. Hosts of mosquitoes in the coastal plain of North Carolina. J. Med. Ent. 25(2):85-93.

141. Issel, C.J. 1973. Isolation of Jamestown Canyon virus (a California group arbovirus) from a white-tailed deer. Am. J. Trop. Med. Hyg. 22(3):414-417.

142. Jakob, W.L., D.B. Francy and R.A. LeBrun. 1986. *Culex tarsalis* in Rhode Island. J. Am. Mosq. Control Assoc. 2(1):98-99.

143. Jakob, W.L., D.B. Francy, J. Mullinix and S.A. Taylor. 1980. Further studies on the *Culex pipiens* complex in Memphis, Tennessee. Mosq. Syst. 12:371-376.

144. Jennings, W.L., A.L. Lewis, G.E. Sather, M.McD. Hammon and J.D. Bord. 1968. California-encephalitis-group viruses in Florida rabbits: Report of experimental and sentinel studies. Am. J. Trop. Med. Hyg. 17:781-787.

145. Jensen, T. and R.K. Washino. 1991. An assessment of the biological capacity of a Sacramento Valley population of *Aedes melanimon* to vector arboviruses. Am. J. Trop. Med. Hyg. 44(4):355-363.

146. Jensen, T., V. Kramer and R.K. Washino. 1993. Short-term population dynamics of adult *Aedes dorsalis* (Diptera: Culicidae) in a northern California tidal marsh. J. Med. Ent. 30(2):374-377.

147. Joseph, S.R. and W.E. Bickley. 1969. *Culiseta melanura* (Coquillett) on the eastern shore of Maryland (Diptera: Culicidae). Univ. Maryland Agr. Exp. Sta. Bull. A-161:1-84.

148. Kappus, K.D., T.P. Monath, R.M. Kaminski and C.H. Calisher. 1983. Reported encephalitis associated with California serogroup virus infections in the United States, 1963-1981. pp. 31-41. In: C.H Calisher and W.H. Thompson (eds.) California Serogroup Viruses. Alan R. Liss, Inc., New York.

149. Karabatsos, N. (ed.). 1985. International Catalogue of Arboviruses (3d ed.). Am. Soc. Trop. Med. Hyg., San Antonio, Texas.

150. Karabatsos, N., A.T.C. Bourke and J.R. Henderson. 1963. Antigenic variation among strains of western equine encephalomyelitis virus. Am. J. Trop. Med. 12:408-412.

151. Kay, B.H. and E.T. Bulfin. 1977. Modification of a livestock crush into a stable trap for mosquito collection. J. Med. Ent. 13(4-5):515-516.

152. Kissling, R.E., R.W. Chamberlain, D.B. Nelson and D.D. Stamm. 1955. Studies on the North American arthropod-borne encephalitides. VIII. Equine encephalitis studies in Louisiana. Am. J. Hyg. 62(3):233-254.

153. Kline, D.L., J.R. Wood and J.A. Cornell. 1991. Interactive effects of 1-octen-3-ol and carbon dioxide on mosquito (Diptera: Culicidae) surveillance and control. J. Med. Ent. 28(2):254-258.

154. Knight, K.L. and A. Stone. 1977. A catalog of the mosquitoes of the world (Diptera: Culicidae) (2d ed.). Thos. Say Found'n., Vol. VI. Ent. Soc. Am., College Park, MD. 611 pp.

155. Kuno, G., D.J. Gubler, M. Velez and A. Oliver. 1985. Comparative sensitivity of three mosquito cell lines for isolation of dengue viruses. Bull. Wld. Hlth. Org. 63(2):279-286.

156. LaMotte, L.D., G.T. Crane, R.B. Shriner and L.J. Kirk. 1967. Use of adult chickens as arbovirus sentinels. I. Viremia and persistence of antibody in experimentally inoculated adult chickens. Am. J. Trop. Med. & Hyg. 16(3):348-356.

157. Lanciotti, R.S., C.H. Calisher, D.J. Gubler, G.-J. Chang and A.V. Vorndam. 1992. Rapid detection and typing of dengue viruses from clinical samples by using reverse transcriptase-polymerase chain reaction. J. Clin. Micro. 30:545-551.

158. Landry, S.V. and G.R. DeFoliart. 1986. Attraction of *Aedes triseriatus* to carbon dioxide. J. Am. Mosq. Control Assoc. 2(3):355-357.

159. Landry, S.V. , G.R. DeFoliart and D.B. Hogg. 1988. Adult body size and survivorship in a field population of *Aedes triseriatus*. J. Am. Mosq. Control Assoc. 4(2):121-128.

160. LaSalle, M.W. and M.E. Dakin. 1982. Dispersal of *Culex salinarius* in southwestern Louisiana. Mosq. News 42(4):543-550.

161. LeDuc, J.W. 1979. The ecology of California group viruses. J. Med. Ent. 16(1):1-17.

162. LeDuc, J.W., W. Suyemoto, B.F. Eldridge and E.S. Saugstad. 1972. Ecology of arboviruses in a Maryland freshwater swamp. II. Blood feeding patterns of potential mosquito vectors. Am. J. Epi. 96:123-128.

163. Lomnicki, A. 1988. Population Ecology of Individuals. Princeton Univ. Press., Princeton, NJ. 223 pp.

164. Loor, K.A. and G.R. DeFoliart. 1969. An oviposition trap for detecting the presence of *Aedes triseriatus* (Say). Mosq. News 29(3):487-488.

165. Lord, R.D., C.H. Calisher, W.A. Chappell, W.R. Metzger, and G.W. Gischer. 1974. Urban St. Louis encephalitis surveillance through wild birds. Am. J. Epidemiol. 99:360-363.

166. Lorenz, L.H., T.W. Scott, R.A. Anderson, J.D. Edman, W.J. Crans and S.D. Costa. 1990. The relationship between size and parity status of field collected *Culiseta melanura*. J. Am. Mosq. Control Assoc. 6(3):433-440.

167. Lounibos, L.P. and R.L. Escher. 1983. Seasonality and sampling of *Coquillettidia perturbans* (Diptera: Culicidae) in south Florida. Env. Ent. 12(4):1087-1093.

168. Magnarelli, L.A. 1977. Physiological age of mosquitoes (Diptera: Culicidae) and observations on partial blood-feeding. J. Med. Ent. 13(4-5):445-450.

169. Magoon, E.H. 1935. A portable stable trap for capturing mosquitoes. Bull Ent. Res. 26:363-369.

170. Main, A.J., S.E. Brown and R.C. Wallis. Arbovirus surveillance in Connecticut. II. California serogroup. Mosq. News 39(3):552-559.

171. Matsumoto, B.M. and H.K. Maxfield. 1985. A comparison of female *Culiseta melanura* captured in New Jersey and CDC light traps in southeastern Massachusetts. J. Am. Mosq. Control Assoc. 1(1):90-91.

172. McClure, E. 1984. Bird Banding. Boxwood Press, Pacific Grove, California. 341 pp.

173. McHugh, C.P. and R.K. Washino. 1986. Survivorship and gonocycle length of *Anopheles freeborni* and *Culex tarsalis* in the Sacramento Valley of California. Proc. Calif. Mosq. Vector Control Assoc. 54:133-135.

174. McKiel, J.A., R.R. Hall and V.F. Newhouse. 1966. Viruses of the California encephalitis complex in indicator rabbits. Am. J. Trop. Med. Hyg. 15:98-102.

175. McLean, R.G. 1991. Arboviruses of wild birds and mammals. Bull. Soc. Vector Ecol. 16:3-16.

176. McLean, R.G. and G.S. Bowen. 1980. Vertebrate hosts. Ch. 8 (pp. 381-450). In: T.P. Monath (ed.) St. Louis Encephalitis. Am. Pub. Hlth. Assoc., Washington, DC.

177. McLean, R.G., G. Frier, G.L. Parham, D.B. Francy, T.P. Monath, E.G. Campos, A. Therrien, J. Kerschner and C.H. Calisher. 1985. Investigations of the vertebrate hosts of eastern equine encephalitis during an epizootic in Michigan, 1980. Am. J. Trop. Med. Hyg. 34(6):1190-1202.

178. McLean, R.G., J. Mullenix. J. Kerschner, and J. Hamm. 1983. The house sparrow (*Passer domesticus)* as a sentinel for St. Louis encephalitis virus. Am. J. Trop. Med. Hyg. 32: 1120-1129.

179. McLean, R.G., R.B. Shriner, L.J. Kirk, and D.J. Muth. 1989. Western equine encephalitis in avian populations in North Dakota, 1975. J. Wildl. Dis. 25: 481-489.

180. McLean, R.G., J.P. Webb, E.G. Campos, J. Gruwell, D.B. Francy, D. Womeldorf, C.M. Myers, T.H. Work, and M. Jozan. 1988. Antibody prevalence of St. Louis encephalitis virus in avian hosts in Los Angeles, California, 1986. J. Am. Mosq. Control Assoc. 4: 524-528.

181. McManus, M.L. 1988. Weather, behaviour and insect dispersal. Mem. Ent. Soc. Can. 146:71-94.

182. Meek, C.L.and G.R. Hayes (eds.). n.d. Mosquito Control Training Manual. Louisiana Mosq. Control Assoc. 152 pp.

183. Meyer, R.P., R.K. Washino and T.L. McKenzie. 1982. Studies on the biology of *Culiseta inornata* (Diptera: Culicidae) in three regions of central California, USA. J. Med. Ent. 19(5):558-568.

184. Meyer, R.P., R.K. Washino, T.L. McKenzie and C.K. Fukushima. 1984. Comparison of three methods for collecting adult mosquitoes associated with ricefield and irrigated pasture habitats in northern California. Mosq. News 44(3):315-320.

185. Milby, M.M. 1985. Predicting *Culex tarsalis* abundance in Kern County. Proc. Papers Calif. Mosq. Vect. Control Assoc. 52:153-155.

186. Mitchell, C.J. 1977. Arthropod-borne encephalitis viruses and water resource developments. Cah. O.R.S.T.O.M., Ser. Ent. Med. et Parasit. (Paris). 15(3):241-250.

187. Mitchell, C.J. 1991. Vector competence of North and South American strains of *Aedes albopictus* for certain arboviruses: A review. J. Am. Mosq. Control Assoc. 7(3):446-451.

188. Mitchell, C.J., R.F. Darsie, T.P. Monath, M.S. Sabattini & J. Daffner. 1985. The use of an animal-baited net trap for collecting mosquitoes during western equine encephalitis investigations in Argentina. J. Am. Mosq. Control Assoc. 1(1):43-47.

189. Mitchell, C.J., D.B. Francy and T.P. Monath. 1980. Arthropod vectors. Ch. 7. In: T.P. Monath (ed.) St. Louis Encephalitis. Am. Pub. Hlth. Assoc., Washington, DC.

190. Mitchell, C.J., R.O. Hayes, P. Holden and T.B. Hughes, Jr. 1973. Nesting activity of the house sparrow in Hale County, Texas, during 1968. Ornithol. Monogr. 14:49-59.

191. Mitchell, C.J., M.L. Niebylski, G.C. Smith, N. Karabatsos, D. Martin, J.-P. Mutebi, G.B. Craig and M.J. Mahler. Isolation of eastern equine encephalitis virus from *Aedes albopictus* in Florida. (To be submitted to Science).

192. Mitchell, C.J., G.C. Smith and B.R. Miller. 1990. Vector competence of *Aedes albopictus* for a newly recognized *Bunyavirus* from mosquitoes collected in Potosi, Missouri. J. Am. Mosq. Control Assoc. 6(3):523-527.

193. Mokgweetsinyana, S.S. 1987. Survival rates of the inland floodwater mosquito *Aedes vexans* (Meigen) in northern Colorado. M.S. Thesis, Colorado State Univ., Ft. Collins, CO.

194. Mokry, J. 1984. Studies on Newfoundland *Aedes* mosquitoes with reference to their reproductive and vector potentials. Mosq. News 44(2):221-227.

195. Monath, T.P. 1979. Arthropod-borne encephalitides in the Americas. Bull. Wld. Hlth. Org. 57(4):513-533.

196. Monath, T.P. 1980. Epidemiology (Ch. 6). In: Monath, T.P. (ed.) St. Louis Encephalitis. Am. Pub. Hlth. Assoc., Washington, DC.

197. Monath, T.P. and T.F. Tsai. 1987. St. Louis encephalitis: Lessons from the last decade. Am. J. Trop. Med. Hyg. 37(3s):40s-59s.

198. Moore, C.G. 1963. Seasonal variation in autogeny in *Culex tarsalis* Coq. in northern California. Mosq. News 23(3):238-241.

199. Moore, C.G., D.B. Francy, D.A. Eliason, R.E. Bailey and E.G. Campos. 1990. *Aedes albopictus* and other container-inhabiting mosquitoes in the United States: Results of an eight-city survey. J. Am. Mosq. Control Assoc. 6(2):173-178.

200. Moore, C.G., D.B. Francy, D.A. Eliason, R.E. and T.P. Monath. 1988. *Aedes albopictus* in the United States: Rapid spread of a potential disease vector. J. Am. Mosq. Control Assoc. 4(3):356-361.

201. Moore, C.G., P. Reiter and J.-J. Xu. 1986. Determination of chronological age in *Culex pipiens s.l.*. J. Am. Mosq. Control Assoc. 2(2):204-208.

202. Morris, C.D. 1988. Eastern equine encephalomyelitis. pp. 1-20. In: T.P. Monath (ed.). The Arboviruses: Epidemiology and Ecology, Vol. III. CRC Press, Inc. Boca Raton, FL.

203. Morris, C.D., M.E. Corey, D.E. Emord and J.J. Howard. 1980. Epizootiology of eastern equine encephalomyelitis virus in upstate New York, USA. I. Introduction, demography and natural environment of an endemic focus. J. Med. Ent. 17(5):442-452.

204. Morris, C.D., R.H. Baker, and W.R. Opp (eds.). 1992. H.T. Evans' Florida Mosquito Control Handbook. Florida Mosq. Control Assoc. (Loose-leaf).

205. Morris, C.D. and S. Srihongse. 1978. An evaluation of the hypothesis of transovarial transmission of eastern equine encephalomyelitis virus by *Culiseta melanura*. Am. J. Trop. Med. Hyg. 27(6):1246-1250.

206. Morris, C.D., R.H. Zimmerman and J.D. Edman. 1980. Epizootiology of eastern equine encephalomyelitis virus in upstate New York. II. Population dynamics and vector potential of adult *Culiseta melanura* (Diptera: Culicidae) in relation to distance from breeding site. J. Med. Ent. 17(5):453-465.

207. Morris, C.D., R.H. Zimmerman and L.A. Magnarelli. 1976. The bionomics of *Culiseta melanura* and *Culiseta morsitans dyari* in central New York state (Diptera: Culicidae). Ann. Ent. Soc. Amer. 69(1):101-105.

208. Mulhern, T.D. 1942. New Jersey mechanical trap for mosquito surveys. N.J. Agric. Exp. Sta., Circ. 421. [Reprinted in J. Am. Mosq. Control Assoc. 1(4):411-418; 1985].

209. Murray, R.A., L.A. Habel, K.J. Mackey, H.G. Wallace, B.A. Peck, S.J. Mora, M.M. Ginsberg and R.W. Emmons. 1985. Epidemiologic aspects of the 1984 St. Louis encephalitis epidemic in southern California. Proc. Calif. Mosq. Vector Control Assoc. 53:5-9.

210. Muul, I., B.K. Johnson and B.A. Harrison. 1975. Ecological studies of *Culiseta melanura* (Diptera: Culicidae) in relation to eastern and western equine encephalitis viruses on the eastern shore of Maryland. J. Med. Ent. 11(6):739-748.

211. Nasci, R.S. 1981. A lightweight battery-powered aspirator for collecting resting mosquitoes in the field. Mosq. News 41(4):808-811.

212. Nasci, R.S. 1985. Behavioral ecology of variation in blood-feeding and its effect on mosquito-borne diseases. pp. 293-303. In: Lounibos, L.P., J.R. Rey and J.H. Frank (eds.). Ecology of Mosquitoes: Proceedings of a Workshop. Florida Medical Entomology Laboratory, Vero Beach, FL.

213. Nasci, R.S. and J.D. Edman. 1981. Blood-feeding patterns of *Culiseta melanura* (Diptera: Culicidae) and associated sylvan mosquitoes in southeastern Massachusetts eastern equine encephalitis enzootic foci. J. Med. Ent. 18(6):493-500.

214. Nasci, R.S. and J.D. Edman. 1981. Vertical and temporal flight activity of the mosquito *Culiseta melanura* (Diptera: Culicidae) in southeastern Massachusetts. J. Med. Ent. 18(6):501-504.

215. Nasci, R.S. and J.D. Edman. 1984. *Culiseta melanura* (Diptera: Culicidae): Population structure and nectar feeding in a freshwater swamp and surrounding areas in southeastern Massachusetts, USA. J. Med. Ent. 21(5):567-572.

216. Nayar, J.K. 1982. Bionomics and physiology of *Culex nigripalpus* (Diptera: Culicidae) of Florida: An important vector of diseases. Florida Agr. Exp. Sta. Bull. No. 827. 73 pp.

217. Nayar, J.K., L. Rosen and J.W. Knight. 1986. Experimental vertical transmission of Saint Louis encephalitis virus by Florida mosquitoes. Am. J. Trop. Med. Hyg. 35(6):1296-1301.

218. Neitzel, D.F. and P.R. Grimstad. 1991. Serological evidence of California group and Cache Valley virus infection in Minnesota white-tailed deer. J. Wildl. Dis. 27(2):230-237.

219. Nelson, M.J. 1971. Mosquito studies (Diptera: Culicidae). XXVI. Winter biology of *Culex tarsalis* in Imperial Valley, California. Contr. Am. Ent. Inst. 7:1-56.

220. Nelson, R.L., C.H. Tempelis, W.C. Reeves and M.M. Milby. 1976. Relation of mosquito density to bird:mammal feeding ratios of *Culex tarsalis* in stable traps. Am. J. Trop. Med. Hyg. 25(4):644-654.

221. Newhouse, V.F., R.W. Chamberlain, J.G. Johnson Jr and W.D. Sudia. 1966. Use of dry ice to increase mosquito catches of the CDC miniature lithe trap. Mosq. News 26(1):30-35.

222. Nielsen, L.T. 1964. Swarming and some other habits of *Mansonia perturbans* and *Psorophora ferox.* Behavior 24:67-89. (Quoted by Bohart & Washino 1978)

223. Olson, J.G. 1976. The impact of *Culex tarsalis* population density and physical environmental factors upon mosquito-borne encephalitis in humans and equines in California. Ph.D. dissertation, Univ. Calif., Berkeley.

224. Olson J.G., W.C. Reeves, R.W. Emmons and M.M. Milby. 1979. Correlation of *Culex tarsalis* population indices with the incidence of St. Louis encephalitis and western equine encephalomyelitis in California. Am. J. Trop. Med. Hyg. 28(2):335-343.

225. Parker, B.M. 1988. Photoperiod-induced diapause in a North Carolina strain of *Aedes sollicitans:* Photosensitivity of fully formed and developing embryos. J. Am. Mosq. Control Assoc. 4(1):57-63.

226. Parsons, R.E. 1977. An improved bait trap for mosquito collecting. Mosq. News 37(3):527-528.

227. White, P.S., and S.T.A. Pickett. 1985. Natural disturbance and patch dynamics: An introduction. In: Pickett, S.T.A., and P.S. White. (eds.) The Ecology of Natural Disturbance and Patch Dynamics. Academic Press, Inc. New York, NY.

228. Provost, M.W. 1969. The natural history of *Culex nigripalpus*. In: Florida State Board of Health. St. Louis Encephalitis in Florida. Fla. State Bd. of Hlth. Monogr. No. 12. Jacksonville, FL. pp. 46-62.

229. Pryor, S.C. & J. Daly. 1991. Temporal variation in morphological and genetic characteristics within a hybrid population of *Culex pipiens* (Diptera: Culicidae). J. Med. Ent. 28(4):481-486.

230. Pumpuni, C.B. and E.D. Walker. 1989. Population size and survivorship of adult *Aedes triseriatus* in a scrap tireyard in northern Indiana. J. Am. Mosq. Control Assoc. 5(2):166-172.

231. Rainey, M.B., G.V. Warren, A.D. Hess and J.S. Blackmore. 1962. A sentinel chicken shed and mosquito trap for use in encephalitis field studies. Mosq. News 22(4):337-342.

232. Reeves, W.C. 1971. Mosquito vector and vertebrate host interaction: The key to maintenance of certain arboviruses. pp. 223-230. In: A.M. Fallis (ed.) Ecology and Physiology of Parasites. Univ. Toronto Press, Toronto, Canada.

233. Reeves, W.C. 1990. Epidemiology and control of mosquito-borne arboviruses in California, 1943-1987. Calif. Mosq. Vector Control Assoc., Inc. Sacramento, California.

234. Reeves, W.C. and W. McD. Hammon. 1962. Epidemiology of the arthropod-borne viral encephalitides in Kern County, California, 1943-1952. II. Infection in other vertebrate hosts. Univ. Calif. Press, Berkeley.

235. Reeves, W.C. and M.M. Milby. 1990. Natural infection in arthropod vectors. In: W.C. Reeves (ed.) Epidemiology and Control of Mosquito-borne Arboviruses in California, 1943-1987. Calif. Mosq. Vector Control Assoc., Sacramento, Calif. pp 128-144.

236. Reeves, W.C. and M.M. Milby. 1990. Strategies for vector control. pp. 383-430. In: W.C. Reeves (ed.) Epidemiology and Control of Mosquito-borne Arboviruses in California, 1943-1987. Calif. Mosq. Vector Control Assoc., Sacramento, Calif.

237. Reeves, W.C., M.M. Milby and W.K. Reisen. 1990. Development of a statewide arbovirus surveillance program and models of vector populations and virus transmission. pp. 431-459. In: W.C. Reeves (ed.) Epidemiology and Control of Mosquito-borne Arboviruses in California, 1943-1987. Calif. Mosq. Vector Control Assoc., Sacramento, Calif.

238. Reisen, W.K., J.L. Hardy and S.B. Presser. 1992. Evaluation of domestic pigeons as sentinels for detecting arbovirus activity in southern California. Am. J. Trop. Med. Hyg. 46(1):69-79.

239. Reisen, W.K., R.P. Meyer and M.M. Milby. 1989. Studies on the seasonality of *Culiseta inornata* in Kern County, California. J. Am. Mosq. Control Assoc. 5(2):183-195.

240. Reisen, W.K., M.M. Milby, R.P. Meyer, A.R. Pfuntner, J. Spoehel, J.E. Hazelrigg and J.P. Webb, Jr. 1991. Mark-release-recapture studies with *Culex* mosquitoes (Diptera: Culicidae) in southern California. J. Med. Ent. 28(3):357-371.

241. Reisen, W.K. and T.P. Monath. 1988. Western equine encephalomyelitis. pp. 89-137. In: T.P. Monath (ed.) The Arboviruses: Epidemiology and Ecology, vol. 4. CRC Press, Inc., Boca Raton, FL.

242. Reisen, W.K. and A.R. Pfuntner. 1987. Effectiveness of five methods for sampling adult *Culex* mosquitoes in rural and urban habitats in San Bernardino County, California. J. Am. Mosq. Control Assoc. 3(4):601-606.

243. Reisen, W.K. and W.C. Reeves. 1990. Bionomics and ecology of *Culex tarsalis* and other potential mosquito vector species. Ch. VI. In: W.C. Reeves (ed.) Epidemiology and Control of Mosquito-borne Arboviruses in California, 1943-1987. Calif. Mosq. Vector Control Assoc., Sacramento, Calif. pp 254-329.

244. Reiter, P. 1983. A portable, battery-powered trap for collecting gravid *Culex* mosquitoes. Mosq. News 43:496-498.

245. Reiter, P. 1986. A standardized procedure for the quantitative surveillance of certain *Culex* mosquitoes by egg raft collection. J. Am. Mosq. Control Assoc. 2(2):219-221.

246. Reiter, P. 1987. A revised version of the CDC gravid mosquito trap. J. Am. Mosq. Control Assoc. 3(2):325-327.

247. Reiter, P. 1988. Weather, vector biology, and arboviral recrudescence (Ch. 9). In: Monath. T.P. (ed.) The Arboviruses: Epidemiology and Ecology. Vol. I. CRC Press. Boca Raton, FL.

248. Reiter, P., W.L. Jakob, D.B. Francy & J.B. Mullenix. 1986. Evaluation of the CDC gravid trap for the surveillance of St. Louis encephalitis vectors in Memphis, Tennessee. J. Am. Mosq. Control Assoc. 2(2):209-211.

249. Robertson, R.C. and S.M.K. Hu. 1935. The tiger mosquito in Shanghai. China J. 23:299-306.

250. Rosen, L. and D.J. Gubler. 1974. The use of mosquitoes to detect and propagate dengue viruses. Am. J. Trop. Med. Hyg. 23(6):1153-1160.

251. Rupp, H.R. 1977. *Culiseta melanura* larvae in tires--a recurring phenomenon? Mosq. News 37(4):772-3.

252. Schlein, Y. and N.G. Gratz. 1972. Age determination of some flies and mosquitoes by daily growth layers of skeletal apodemes. Bull. Wld. Hlth. Org. 47:71-76.

253. Schreiber, E.T., M.S. Mulla, J.D. Chaney and M.S. Dhillon. 1988. Dispersal of *Culex quinquefasciatus* from a dairy in southern California. J. Am. Mosq. Control Assoc. 4(3):300-304.

254. Scott, T.W. and S.C. Weaver. 1989. Eastern equine encephalomyelitis virus: Epidemiology and evolution of mosquito transmission. Adv. Virus Res. 37:277-328.

255. Scotton, G.L. and R.C. Axtell. 1979. *Aedes taeniorhynchus* and *Ae. sollicitans* (Diptera: Culicidae) ovipositing on coastal dredge spoil. Mosq. News 39(1):97-110.

256. Sellers, R.F. and A.R. Maarouf. 1988. Impact of climate on western equine encephalitis in Manitoba, Minnesota and North Dakota, 1980-1983. Epidemiol. Infect. 101:511-535.

257. Sellers, R.F. and A.R. Maarouf. 1990. Trajectory analysis of winds and eastern equine encephalitis in the USA, 1980-85. Epidemiol. Inf. 104:329-343.

258. Service, M.W. 1976. Mosquito Ecology, Field Sampling Methods. J. Wiley & Sons, New York. 583 pp.

259. Service, M.W. 1985. Population dynamics and mortalities of mosquito preadults. pp. 185-201. In: Lounibos, L.P., J.R. Rey & J.H. Frank (eds.) Ecology of Mosquitoes: Proceedings of a Workshop. Florida Medical Entomology Laboratory, Vero Beach, FL.

260. Shemanchuk, J.A. 1969. Epidemiology of western encephalitis in Alberta: Response of natural populations of mosquitoes to avian host. J. Med. Ent. 6:269-275.

261. Showers, W.B., F. Whitford, R.B. Smelser, A.J. Keaster, J.F. Robinson, J.D. Lopez and S.E. Taylor. 1989. Direct evidence for meteorologically driven long-range dispersal of an economically important moth. Ecology 70(4):987-992.

262. Shroyer, D.A. *Aedes albopictus* and arboviruses: A concise review of the literature. J. Am. Mosq. Control Assoc. 2(4):424-428.

263. Sinsko, M.J. & G.B. Craig, Jr. 1981. Dynamics of an isolated population of *Aedes triseriatus* (Diptera: Culicidae). II. Factors affecting productivity of immature stages. J. Med. Ent. 18(4):279-283.

264. Sinsko, M.J. and P.R. Grimstad. 1977. Habitat separation by differential vertical oviposition of two treehole *Aedes* in Indiana. Env. Ent. 6(3):485-487.

265. Sirivanakarn, S. 1976. Medical entomology studies. III. A revision of the subgenus *Culex* in the Oriental Region. (Diptera: Culicidae). Contr. Am. Ent. Inst. 12(2):1-272.

266. Slaff, M. and W.J. Crans. 1981. The activity and physiological status of pre- and posthibernating *Culex salinarius* (Diptera: Culicidae) populations. J. Med. Ent. 18(1):65-68.

267. Slaff, M. and W.J. Crans. 1981. The host-seeking activity of *Culex salinarius*. Mosq. News 41(3):443-447.

268. Slaff, M. and W.J. Crans. 1982. Impounded water as a major producer of *Culex salinarius* (Diptera: Culicidae) in coastal areas of New Jersey. J. Med. Ent. 19:185-190.

269. Smith, G.C., D.B. Francy, E.G. Campos, P. Katona and C.H. Calisher. 1983. Correlation between human cases and antibody prevalence in house sparrows during a focal outbreak of St. Louis encephalitis in Mississippi, 1979. Mosq. News 43:322-325.

270. Spadoni, R.D., R.L. Nelson and W.C. Reeves. 1974. Seasonal occurrence, egg production, and blood-feeding activity of autogenous *Culex tarsalis*. Ann. Ent. Soc. Am. 67:895-902.

271. Spielman, A. 1964. Swamp mosquito, *Culiseta melanura:* Occurrence in an urban habitat. Science 143:361-362.

272. Sprance, H.E. 1981. Experimental evidence against the transovarial transmission of eastern equine encephalitis virus in *Culiseta melanura.* Mosq. News 41(1):168-173.

273. Sprenger, D. and T. Wuithiranyagool. 1986. The discovery and distribution of *Aedes albopictus* in Harris County, Texas. J. Am. Mosq. Control Assoc. 2:217-219.

274. Stamm, D.D. 1958. Studies on the ecology of equine encephalomyelitis. Am. J. Pub. Hlth. 48:328-335.

275. Stamm, D.D. 1968. Arbovirus studies in birds in south Alabama, 1959-1960. Am. J. Epidemiol. 87:127-137.

276. Stamm, D.D., R.W. Chamberlain and W.D. Sudia. 1962. Arbovirus studies in south Alabama, 1957-1958. Am. J. Hyg. 76(1):61-81.

277. Sudia, W.D. and R.W. Chamberlain. 1962. Battery-operated light trap, an improved model. Mosq. News 22:126-129.

278. Sudia, W.D. and R.W. Chamberlain. 1964. Experimental infection of *Culex nigripalpus* Theobald with the virus of St. Louis encephalitis. Am. J. Trop. Med. Hyg. 13(3):469-471.

279. Sudia, W.D., R.D. Lord and R.O. Hayes. 1970. Collection and processing of vertebrate specimens for arbovirus studies. Communicable Disease Center, Atlanta, GA, 65 pp.

280. Sudia, W.D., V.F. Newhouse, C.H. Calisher and R.W. Chamberlain. 1971. California group arboviruses: Isolations from mosquitoes in North America. Mosq. News 31(4):576-600.

281. Sutherland, W.S. and D.W. Brassard. 1987. EPA list of insects and other organisms (For use with the EPA Index to Pesticide Chemicals: Their Uses and Limitations on Pesticide Labeling and NPIRS). Unpub. Doc., Animal Biology & Entomology Sec., Sci. Suppt. Br., Benefits & Use Div., Off. Pestic. Progs, USEPA, Washington, DC.

282. Taylor, D.J., A.L. Lewis, J.D. Edman and W.L. Jennings. 1971. California group arboviruses in Florida: Host-vector relationships. Am. J. Trop. Med. Hyg. 20:139-145.

283. Taylor, D.W., K.E. Meadows, N.J. Schneider, J.A. Mulrennan and E. Buff. 1969. St. Louis encephalitis virus and *Culex nigripalpus* in Florida - review of field observations and laboratory transmission experiments. pp. 34-45. In: St. Louis Encephalitis in Florida. Fla. State Bd. of Health Monogr. No. 12.

284. Tempelis, C.H. 1975. Host-feeding patterns of mosquitoes, with a review of advances in analysis of blood meals by serology. J. Med. Ent. 11(6):635-653.

285. Tsai, T.F. 1992. Arboviral diseases of North America. In Feign, R.D. and J.D. Cherry (eds). Pediatric Infectious Diseases (3d ed.). W.B. Saunders, Philadelphia, PA (In Press).

286. Tsai, T.F., W.B. Cobb, R.A. Bolin, N.J. Gilman, G.C. Smith, R.E. Bailey, J.D. Poland, J.J. Doran, J.K. Emerson and K.J. Lampert. 1987. Epidemiologic aspects of a St. Louis encephalitis outbreak in Mesa County, Colorado. Am. J. Epidemiol. 126:460-473.

287. Tsai, T.F., C.M. Happ, R.A. Bolin, M. Montoya, E. Campos, D.B. Francy, R.A. Hawkes and J.T. Roehrig. 1988. Stability of St. Louis encephalitis viral antigen detected by enzyme immunoassay in infected mosquitoes. J. Clin. Microbiol. 26(12):2620-2625.

288. Tsai, T.F. and C.J. Mitchell. 1988. St. Louis encephalitis (Ch. 42). In: Monath, T.P. (ed.) The Arboviruses: Epidemiology and Ecology. Vol IV, pp. 113-143. CRC Press, Boca Raton, FL.

289. Tsai, T.F., G.C. Smith, M. Ndukwu, W.L. Jakob, C.M. Happ, L.J. Kirk, D.B. Francy and K.J. Lampert. 1988. Entomologic studies after a St. Louis encephalitis epidemic in Grand Junction, Colorado. Am. J. Epidemiol. 128:285-297.

290. Tsai, T.F., G.C. Smith, C.M. Happ, L.J. Kirk, W.L. Jakob, R.A. Bolin, D.B. Francy and K.J. Lampert. 1989. Surveillance of St. Louis encephalitis virus vectors in Grand Junction, Colorado, in 1987.

291. Turell, M.J., J.L. Hardy and W.C. Reeves. 1982. Stabilized infection of California encephalitis virus in *Aedes dorsalis*, and its implications for viral maintenance in nature. Am. J. Trop. Med. Hyg. 31(6):1252-1259.

292. Turell, M.J. and J.W. LeDuc. 1983. The role of mosquitoes in the natural history of California serogroup viruses. pp. 43-55. In: Calisher, C.H. (ed.). California Serogroup Viruses. Alan R. Liss, New York.

293. Walker, E.D., R.S. Copeland, S.L. Paulson and L.E. Munstermann. 1987. Adult survivorship, population density, and body size in sympatric populations of *Aedes triseriatus* and *Aedes hendersoni* (Diptera: Culicidae). J. Med. Ent. 24(4):485-493.

294. Wallis, R.C., J.J. Howard, A.J. Main, Jr., C. Frazier and C. Hayes. 1974. An increase of *Culiseta melanura* coinciding with an epizootic of eastern equine encephalitis in Connecticut. Mosq. News 34:63-65.

295. Walsh J. 1983. California's Mosquito-borne Encephalitis Virus Surveillance and Control Program. Calif. Dept. of Health Services, Sacramento. 26 pp.

296. Washino, R.K., R.L. Nelson, W.C. Reeves, R.P. Scrivani and C.H. Tempelis. 1962. Studies on *Culiseta inornata* as a possible vector of encephalitis viruses in California. Mosq. News 22(3):268-274.

297. Watts, D.M., J.W. LeDuc, C.L. Bailey, J.M. Dalrymple and T.P. Gargan II. 1982. Serologic evidence of Jamestown Canyon and Keystone virus infection in vertebrates of the DelMarVa Peninsula. Am. J. Trop. Med. Hyg. 31(6):1245-1251.

298. Watts, D.M., S. Pantuwatana, G.R. DeFoliart, T.M. Yuill and W.H. Thompson. 1973. Transovarial transmission of LaCrosse virus (California encephalitis group) in the mosquito, *Aedes triseriatus*. Science 182:1140-1141.

299. Watts, D.M., W.H. Thompson, T.M. Yuill, G.R. DeFoliart and R.P. Hanson. 1979. Overwintering of LaCrosse virus in *Aedes triseriatus*. Am. J. Trop. Med. Hyg. 23:694-700.

300. Wilton, D.P. 1968. Oviposition site selection by the tree-hole mosquito, *Aedes triseriatus* (Say). J. Med. Ent. 5(2):189-194.

301. Wilton, D.P. 1981. Light-trap response and the DV/D ratio in the *Culex pipiens* complex (Diptera: Culicidae). J. Med. Ent. 18(4):284-288.

302. Wilton, D.P., R.F. Darsie & R. Story. 1985. Trials with portable screen rooms modified for use as animal-baited net traps for mosquito collection. J. Am. Mosq. Control Assoc. 1(2):223-226.

303. Wright, R.E. and G.R. DeFoliart. 1970. Associations of Wisconsin mosquitoes and woodland vertebrate hosts. Ann. Ent. Soc. Am. 63(3):777-786.

304. Work, T.H. 1983. Emergence of California encephalitis as a continental epidemiological, virological, and human disease problem. pp. 205-214. In: C.H. Calisher, and W.H. Thompson (eds.) California Serogroup Viruses. Alan R. Liss, Inc., New York.

305. Yuill, T.M. 1983. The role of mammals in the maintenance and dissemination of La Crosse virus. pp. 77-87. In: Calisher, C.H. (ed.) California Serogroup Viruses. Alan R. Liss, New York.

306. Zavortink, T.J. 1972. Mosquito studies (Diptera, Culicidae). XXVIII. The New World species formerly placed in *Aedes* (*Finlayia*). Contr. Am. Ent. Inst. 8(3):1-206.

CHIKUNGUNYA VIRUS

Guidance for U.S. state and territorial health departments

Chikungunya virus disease case investigation, diagnosis, and response for U.S. state and territorial health departments

Scenarios

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Information and contacts

The CDC Arboviral Diseases Branch in Fort Collins, Colorado is responsible for chikungunya surveillance, response, and diagnostic testing.

More information is available at http://www.cdc.gov/chikungunya/.

For questions or reporting, please contact the Arboviral Diseases Branch on call epidemiologist at 970-221-6400.

Scenario 1: Patient with clinical illness but chikungunya virus testing not yet performed

1. Obtain or confirm initial clinical and epidemiologic data
    a. Demographics (age, sex, place of residence)
    b. Clinical symptoms
    c. Date of illness onset
    d. Hospitalization
    e. Travel history in 2 weeks prior to illness onset

2. Establish if the patient has a clinically compatible illness of fever and polyarthralgia or polyarthritis
    a. Clinically compatible illness: Continue investigation for possible chikungunya virus or other arboviral infections.
    b. No clinically compatible illness: Determine if there are other reasons to continue investigation for possible chikungunya virus or other arboviral infections.

3. Assess for possible travel-associated versus locally-acquired infection
    a. Recent travel: Determine the specific dates and location of travel in the 2 weeks prior to illness onset. If recent travel to area with no known local transmission, notify CDC Arboviral Diseases Branch.
    b. No recent travel: Determine if the local health department or healthcare provider is aware of other similar cases in the area or among contacts of the patient. If concern of local transmission in a new area, notify CDC Arboviral Diseases Branch.

4. Assess risk of being viremic while in United States
    a. No travel outside the United States
    b. Onset of symptoms within the last 7 days, or
    c. Returned to the United States <7 days after illness onset

5. If risk of viremia, assess and mitigate risk of local transmission
    a. Recommend the case-patient stay in air conditioned or screened accommodations during the first week of illness and reduce mosquito breeding sites in and around the patient's home
    b. Work with local public health officials and healthcare personnel to perform enhanced surveillance for people with similar illnesses in the community
    c. Consult with local health department, vector control agencies, and/or CDC Arboviral Diseases Branch to assess whether *Aedes aegypti* or *Ae. albopictus* mosquitoes are likely present and active in the local area, and determine if vector control and mosquito trapping/testing should be considered in the area

6. Ensure laboratory testing is performed for chikungunya and dengue viruses and obtain results [Appendix A]
    a. Positive test results: Complete case investigation [Scenario 2]
    b. Negative test results: Determine if additional testing is needed

Scenario 2: Patient with positive chikungunya virus test results

1. Perform standard case investigation to obtain or confirm clinical and epidemiologic data
    a. Demographics (age, sex, race/ethnicity, place of residence)
    b. Clinical symptoms and syndrome
    c. Date of illness onset
    d. Hospitalization and outcome
    e. Travel history in 2 weeks prior to illness onset
    f. Organ, tissue, or blood donor or recipient
    g. Pregnant or breast feeding
    h. Contacts with similar illness

2. If the patient is a recent organ, tissue (e.g., corneas, skin), or blood donor or recipient
    a. Notify blood or tissue banks
    b. Quarantine remaining co-component blood or tissues
    c. Identify other possibly exposed patients
    d. Notify CDC Arboviral Diseases Branch

3. Assess for possible travel-associated versus locally-acquired infection
    a. <u>Recent travel</u>: Determine the specific dates and location of travel in the 2 weeks prior to illness onset. If recent travel to area with no known local transmission, notify CDC Arboviral Diseases Branch.
    b. <u>No recent travel</u>: Determine if the local health department or healthcare provider is aware of other similar cases in the area or among contacts of the patient. If concern of local transmission in a new area, notify CDC Arboviral Diseases Branch.

4. Assess evidence or risk of being viremic while in United States
    a. Positive RT-PCR or viral culture
    b. No travel outside the United States
    c. Onset of symptoms within the last 7 days
    d. Returned to the United States <7 days after illness onset

5. If evidence or risk of viremia, assess and mitigate risk of local transmission
    a. Recommend the case-patient stay in air conditioned or screened accommodations during the first week of illness and reduce mosquito breeding sites in and around the patient's home
    b. Work with local public health officials and healthcare personnel to perform enhanced surveillance for people with similar illnesses in the community
    c. Consult with local health department, vector control agencies, and/or CDC Arboviral Diseases Branch to assess whether *Aedes aegypti* or *Ae. albopictus* mosquitoes are likely present and active in the local area, and determine if vector control and mosquito trapping/testing should be considered in the area

6. If there is evidence of local transmission
    a. Work with local health department and vector control agencies to determine vector control options
    b. Inform the public of the potential transmission risk and prevention measures
    c. Notify CDC Arboviral Diseases Branch

7. Determine chikungunya case classification [Appendix B]
    a. <u>Confirmed or probable case</u>: Report case to ArboNET
    b. <u>Indeterminate</u>: Decide if additional testing is needed
    c. <u>Not a case:</u> Notify healthcare provider and relevant partners

Appendix A. Diagnostic testing for chikungunya virus

Laboratories that perform chikungunya diagnostic testing (as of June 2014)

- CDC Arboviral Diseases Branch (Fort Collins, CO)
- California, Florida, and New York State Departments of Health
- Focus Diagnostics

Chikungunya virus diagnostic assays*

- Viral culture
- Reverse transcriptase-polymerase chain reaction (RT-PCR)
- Enzyme-linked immunosorbent assay (ELISA) or immunofluorescence assay (IFA) for immunoglobulin (Ig) M or IgG antibodies
- Plaque reduction neutralization test (PRNT)
- Immunohistochemical staining (IHC)

Routine chikungunya virus diagnostic testing performed on serum specimens at CDC

- RT-PCR: ≤5 days after illness onset†
- IgM antibody tests: ≥5 days after illness onset‡

Rationale for testing for both dengue and chikungunya

- Viruses transmitted by same mosquitoes
- Diseases have similar clinical features
- Viruses can circulate in same areas and cause co-infections
- Important to rule out dengue, as proper clinical management can improve outcome
- WHO dengue clinical management guidelines are available at: http://whqlibdoc.who.int/publications/2009/9789241547871_eng.pdf

* *Biosafety in Microbiological and Medical Laboratories (BMBL) 5th edition recommends that chikungunya virus be handled under biosafety level 3 (BSL-3) containment.*

†Viral RNA may be detected in serum for up to 8 days after onset of symptoms.

‡ IgM antibodies are generally first detectable at 4 to 8 days after onset of illness and can persist for months. Serum collected within 8 days of illness onset may not have detectable IgM antibodies and testing should be repeated on a convalescent-phase sample to rule out infection in those with a compatible clinical syndrome.

Appendix B. Chikungunya case definitions and classifications

Confirmed case*

A person with fever or chills as reported by the patient or healthcare provider, absence of a more likely explanation, and one or more of the following laboratory criteria:

- Isolation of virus from, or demonstration of specific viral antigen or nucleic acid in, tissue, blood, or other body fluid, OR
- Four-fold or greater change in virus-specific quantitative antibody titers in paired serum samples, OR
- Virus-specific IgM antibodies in serum with confirmatory neutralizing antibodies in the same or a later specimen

Probable case*

A person with fever or chills as reported by the patient or healthcare provider, absence of a more likely explanation, and virus-specific IgM antibodies in serum but with no other testing

Suspected case

A person with acute onset of fever and severe arthralgia or arthritis not explained by other medical conditions, and who resides or has visited epidemic or endemic areas within 2 weeks before the onset of symptoms.

Indeterminate case

A suspected case without a more likely explanation and negative chikungunya virus testing but no virus-specific IgM or neutralizing antibody testing performed on a serum specimen collected ≥8 days after illness onset

Not a case

A suspected case with negative virus-specific IgM or neutralizing antibodies in serum collected ≥8 days after illness onset or evidence of a more likely explanation for their illness

*Report confirmed and probable cases to ArboNET using the existing CSTE position statement, case definition, data variables, and mechanisms for "Arboviral Diseases, neuroinvasive and non-neuroinvasive". More information is available at **http://wwwn.cdc.gov/nndss/.**

ZIKA

CDC Interim Response Plan

May 2017

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Purpose

This document describes updated guidance and resources from the Centers for Disease Control and Prevention (CDC) for responding to cases of Zika virus infection in the continental United States (CONUS) and Hawaii. This guidance is targeted to state, local, and tribal jurisdictions, which are responsible for responding to Zika virus disease in their communities. This guidance may also be used by jurisdictions in US territories and freely associated states as applicable and adapted as needed. Information is provided to assist jurisdictions in protecting pregnant women and infants and responding to travel-associated, sexually transmitted, and locally acquired Zika virus infections in the United States. CDC encourages jurisdictions to use existing funding to support these activities. There are no additional funds available from CDC at this time to support implementation of this guidance. This document serves as a reference for public health decision-making and is not meant to be prescriptive or comprehensive, as activities and decisions are jurisdiction- and event-specific. The response activities outlined in this plan are based on currently available knowledge about Zika virus, its transmission, and its effects on pregnant women and infants. These activities may change as more is learned about Zika virus.

Zika Virus in the Continental United States

In 2016, non-congenital and congenital Zika virus infection and disease became nationally notifiable conditions in the United States. Cases are reported to ArboNET, the national arboviral surveillance system managed by CDC and state and territorial health departments, using the Council of State and Territorial Epidemiologists (CSTE) approved case definitions for non-congenital and congenital Zika virus infection and disease. CDC provides weekly provisional data on Zika virus disease case counts and presumptive viremic blood donors reported by US states and territories on the CDC Zika Virus Case Counts in the US website. Zika virus disease cases that meet the probable or confirmed CSTE definitions are categorized as acquired through travel to affected areas, presumed local mosquito-borne transmission, or other routes (e.g., sexual, laboratory, or blood-borne transmission).

Zika virus infection during pregnancy may cause birth defects including microcephaly, fetal brain abnormalities, eye abnormalities, hearing loss, and other consequences of central nervous system damage in infants exposed in utero. Pregnancy loss and neonatal deaths have also been reported. To complement notifiable reporting of Zika virus disease, CDC has established the US Zika Pregnancy Registry (USZPR), an enhanced national surveillance system for monitoring the effects of Zika virus infection on pregnant women and their infants. The USZPR includes pregnant women with laboratory evidence of possible Zika virus infection in a maternal, placental, or fetal/infant sample and monitors these women and their infants.

Within CONUS, local mosquito-borne Zika virus transmission was reported by Florida and Texas in 2016. A description of Florida's response and control efforts for local mosquito-borne transmission of Zika virus in Miami-Dade and Broward Counties during June-August 2016 was published in CDC's Morbidity and Mortality Weekly Report (MMWR). Recommendations and guidance for people living in or traveling to areas of the United States with Zika virus transmission are available on the CDC website.

Building on lessons learned in 2016 and feedback from state and local health partners, CDC has revised this document to be more streamlined, added links to existing guidance on CDC's website, and updated critical guidance. Detailed guidance on issuing and removing designations for Zika active transmission (red) areas and Zika cautionary (yellow) areas has been provided. In addition, the guidance for identifying areas at risk for the purpose of blood and tissue safety is now more closely aligned with the guidance for issuing travel and testing guidance.

Definitions

Local Mosquito-borne Transmission

Zika virus infection in a person who has not traveled from an area with Zika virus transmission or had sexual exposure or other known exposure to body fluids of an infected person.

Suspect Case of Local Mosquito-borne Transmission

A person with symptoms or preliminary test results compatible with Zika virus infection who does not have risk factors for Zika virus acquisition through travel, sexual contact, or other known exposure to body fluids and for whom Zika virus test results are pending.

OR

A blood donor with initial donation screening positive for Zika virus and confirmatory test pending, who does not have risk factors for Zika virus acquisition through travel, sexual contact, or other known exposure to body fluids.

Confirmed Local Mosquito-borne Transmission

A person who does not have risk factors for Zika virus acquisition through travel, sexual contact, or other known exposure to body fluids and who tests positive for Zika virus infection per CDC laboratory guidance.

OR

A blood donor who does not have risk factors for Zika acquisition through travel, sexual contact, or other body fluid exposure and who has a positive Zika virus nucleic acid test (NAT) on screening AND confirmation through an approved confirmatory test algorithm.

Confirmed, Multiperson Local Mosquito-borne Transmission

Three or more cases of confirmed local transmission in non-household members with onsets greater than 2 weeks apart (the approximate lifespan of an infected mosquito) and less than 45 days in an area of approximately 1-mile in diameter. Identification of overlapping movement within a 1-mile diameter of multiple people with locally acquired Zika virus infection suggests a common location (e.g., residential neighborhood,

workplace, or other location) for infected mosquito exposure, because the lifetime flight range of the *Aedes aegypti* mosquito vector is approximately 150 meters (approximately 500 ft).

Preparedness

CDC recommends that jurisdictions develop Zika virus action plans to guide preparedness and response activities through a phased, risk-based continuum. The continuum includes support for mosquito season preparedness and graduated action in response to detection of confirmed local mosquito-borne transmission and multiperson local mosquito-borne transmission, if present. Planning should also address activities to occur in subsequent seasons, following the confirmation of multiperson local mosquito-borne transmission.

Local mosquito-borne transmission can only occur when competent *Aedes* species mosquito populations are present within a community. The temperate climate of CONUS limits year-round Zika virus transmission in most locations; the seasonal timing of imported cases greatly influences the potential for local Zika virus transmission (e.g., imported cases during cooler months are less likely to lead to local mosquito-borne transmission). Mosquito season varies by jurisdiction but is typically during the summer months. However, year-round local transmission of Zika virus may be possible in warmer locations. Jurisdictions with competent vectors should conduct an assessment of vector risks and institute vector control activities as indicated.

Surveillance

Case surveillance

All health departments should be prepared to identify and investigate potential Zika cases in travelers, presumptive viremic blood donors, and their sexual contacts. These activities are important to mitigate risk to the community. Because clinicians are integral to the surveillance process, all health departments should take steps to increase healthcare provider awareness of Zika virus and ensure testing of potential cases. In addition, health departments should ensure that appropriate divisions, sections, or other units within their organizations have established lines of communication and are coordinating planning and response efforts, especially maternal and child health and birth defects programs. Vector control programs, which may exist within or outside health departments, should also be included.

The following information should be gathered as quickly as possible when a potential case is identified:

- Basic demographic information (e.g., age, sex, state, and county of residence)
- Clinical symptoms (including fever, rash, conjunctivitis, arthralgia, or evidence of neurologic disorder, such as Guillain-Barré syndrome)
- Illness onset date
- Exposure history (location of travel, dates of travel, partner's clinical information if sexual transmission is suspected, and receipt of any blood, organ, or tissues in previous 28 days)
    - For each confirmed case, dates of symptom onset and exposure to areas with risk of Zika or sexual contacts at risk for Zika virus infection should be closely evaluated to determine whether

local mosquito-borne transmission can be ruled out. (see Zika Virus Infection Case Investigation Form)
  - o Further detailed investigation should be conducted promptly for people who develop illness compatible with Zika virus disease within 28 days of receiving blood products, organs, or tissue because of the potential for Zika virus transmission through transfusion/transplantation.
- Hospitalization, reason for hospitalization, and disposition
- Pregnancy status and related information (e.g., sexual exposure, estimated date of delivery, results of prenatal ultrasound and other testing, outcomes, including pregnancy loss, live birth and any birth defects)
- If the patient is an infant, obtain maternal history as outlined above, including gestational age during pregnancy at the time of exposure and at birth. Also collect laboratory test results (maternal, infant, or placental), infant physical exam, and imaging findings, including microcephaly, intracranial calcifications, other neurologic abnormalities and birth defects and follow up on growth and development milestones, as appropriate.
- History of blood or tissue donation

Tools and resources to assist health departments in conducting epidemiologic investigations can be found on CDC's website.

While interacting with the patient and family, reinforce the steps necessary to avoid exposure to local mosquito populations to prevent transmission (e.g., stay indoors in screened, air-conditioned rooms during the first week of illness, use Environmental Protection Agency (EPA)-registered insect repellent, and perform mosquito reduction activities around home).

States and blood collection establishments should work together to ensure prompt communication from the blood center to the health department of any positive results identified in blood donors. It is possible that either local transmission cases or travel-associated cases could be identified through blood screening.

Pregnancy and birth defects surveillance

CDC is ready to immediately assist jurisdictions with confirmed local mosquito-borne transmission of Zika virus to protect and educate pregnant women, track cases of Zika infections during pregnancy and infant outcomes, and assist with provider outreach and education. State, local, and territorial public health programs are encouraged to collaborate with the US Zika Pregnancy Registry (USZPR), an active population-based surveillance system that monitors the effects of Zika virus infection during pregnancy on women and their infants. Data from the USZPR are used to update clinical evaluation and management of pregnant women and infants.

Whether symptomatic or asymptomatic, pregnant women with possible Zika virus infection and their infants should be reported to USZPR in as timely a manner as possible. Infants in whom Zika is diagnosed after birth should also be promptly reported, along with their mothers. Clinical information about the pregnancy as well as infant outcome data are tracked as part of USZPR surveillance.

Establishing and maintaining pregnancy and birth defects surveillance capacity is important before and during local mosquito-borne transmission. Jurisdictions should ensure that maternal and child health and birth defects programs are integrated into Zika virus planning and response activities and should ensure that clinicians caring for pregnant women and infants are aware of Zika risks, laboratory test availability, surveillance reporting, and clinical guidance.

Enhanced surveillance in areas at risk for mosquito-borne transmission

In locations with competent mosquito vectors and travel-associated Zika virus disease cases, health departments should consider implementing enhanced surveillance for Zika virus disease when the mosquitoes are present and active and before identifying a first case of local mosquito-borne transmission. The appropriate geographic scope and intensity of such increased surveillance depends on local circumstances, such as history of previous local dengue or chikungunya virus transmission, population density, anticipated mosquito abundance, locations of recent travel-associated cases, local travel patterns (i.e., areas known to have a high number of travelers to affected areas, or areas with previously identified cases of travel-associated dengue and chikungunya), and other risk factors (e.g., lack of air conditioning or screens). Enhanced surveillance near the area(s) of likely exposure should also be conducted when a confirmed case of local transmission is reported. CDC is available to provide additional guidance to state, local, and tribal jurisdictions as requested. Contact CDC's Emergency Operations Center at 770-488-7100 or eocreport@cdc.gov for assistance.

Jurisdictions should take the following steps to quickly detect and report local mosquito-borne transmission:

1. Ensure diagnostic testing is available and communicate with providers about local testing goals.
   - Ensure processes for laboratory test ordering and approval (from public health laboratories [PHLs]) are in place and that providers are aware of PHLs and commercial laboratory diagnostic testing options.
   - Ensure timely specimen transport and testing for suspected local transmission cases and plans for test confirmation if there is a positive result.
   - Ensure any changes in guidance on laboratory testing or interpretation of results are communicated promptly through appropriate public health channels to reach clinicians.
   - Emphasize outreach to healthcare providers caring for pregnant women and infants to ensure they are aware of how to obtain Zika virus testing when indicated.
   - Ensure a mechanism exists for timely reporting of results to providers, particularly those caring for pregnant women.
2. Increase surveillance for Zika virus disease in areas with confirmed travel-associated cases and competent vector activity to identify possible cases of local transmission.
   - Interview household members of confirmed travel-associated cases, conduct testing of anyone with symptoms consistent with Zika virus disease, and inform household members to notify public health authorities if symptoms develop.
     - Whenever possible, follow up on households that had a travel-associated case with onset of symptoms 14-21 days earlier to ascertain if any additional household members developed symptoms that could indicate local transmission, and to provide or facilitate testing for any newly symptomatic people.
     - Deliver prevention and early detection messages to nearby households.

- Increase outreach to local healthcare providers, including infectious disease specialists, obstetricians and gynecologists, maternal-fetal medicine specialists, and pediatricians.
- Develop tools and processes to ensure providers can report clinically compatible cases, particularly pregnant women with prenatal findings consistent with congenital Zika syndrome and infants with birth defects consistent with congenital Zika syndrome to state or local public health officials.
- Contact local laboratories that perform Zika virus testing to monitor the number and geographic location of additional suspect cases and any preliminary positive results, to reconcile with reports from public health departments, to assess testing volume and to ensure laboratories are aware of public health reporting requirements.
- Conduct syndromic surveillance using data from healthcare facilities, including emergency departments, to detect early increases in illnesses that could be Zika virus disease, wherever possible.
- Consider implementing sentinel surveillance in areas at higher risk for local mosquito-borne transmission.
    - Rapidly identifying suspect local mosquito-borne transmission cases in areas with confirmed travel-related cases and potential for mosquito-borne transmission requires timely testing of patients with illnesses highly suggestive of Zika virus disease (e.g., people who have two or more of the four primary clinical signs/symptoms: rash, fever, arthralgia, or conjunctivitis), but who lack known travel-related exposures.
- Consider implementing event-based surveillance for clusters of rash illness.
    - Educate and enlist providers to be vigilant for unexplained clusters of rash illness, to report the finding to public health, and to conduct further investigation and testing for Zika virus disease. This is especially important if some patients have additional symptoms (e.g., fever, arthralgia, or conjunctivitis), or if the cluster involves adults, where rash illnesses may be less common.

3. Develop standing communication channels with vector control officials to share vital information and coordinate surveillance and vector control efforts.
    - State and local health departments should coordinate closely with local vector control districts to ensure vector control personnel are rapidly informed of any confirmed Zika virus infection in their jurisdiction.

Vector Control

The goal of vector control is to suppress *Aedes aegypti* and *Aedes albopictus* mosquito populations in a coordinated and effective manner to prevent or interrupt Zika virus transmission. CDC has developed guidelines on the Surveillance and Control of *Aedes aegypti* and *Aedes albopictus* in the United States. The magnitude of activities used in a vector control response will depend on the extent of mosquito-borne transmission, as measured by the number of Zika cases and their geographic and temporal distribution.

- Control activities that target both adult and larval mosquitoes will be necessary to prevent or interrupt Zika virus transmission by mosquitoes.
- The methods used around a single case may be accomplished with intensive sanitation and limited adulticiding delivered with backpack spraying. More widespread transmission may require equipment (i.e., trucks or aircraft) that can deliver larvicides and adulticides over a much broader area in a timely and effective manner.

- Any vector control should be guided by robust mosquito surveillance to evaluate the effectiveness of interventions.
- The American Mosquito Control Association, through funding provided by CDC, has updated its Best Practices for Integrated Mosquito Management.

CDC has also developed a database for the collection of surveillance information on the distribution, abundance, and insecticide resistance status of *Aedes aegypti* and *Aedes albopictus*. This information is useful to inform strategy and resource allocation for the control of these mosquito vectors. Additional guidance on Zika vector control in the Continental United States can be found here.

Blood and Tissue Safety

The US Food and Drug Administration (FDA) issued updated guidance for industry to reduce the risk of transfusion-transmission of Zika virus in August 2016. These recommendations call for blood collection establishments in **all** states and US territories to screen individual units of donated whole blood and blood components with a Zika virus screening test authorized for use by FDA under an investigational new drug (IND) application or with a licensed test when available. Alternatively, an FDA-approved pathogen-reduction device may be used for plasma and certain platelet products. FDA has also issued guidance for reducing the risk of Zika virus transmission by human cell and tissue products. For organ transplants, the Organ Procurement and Transplantation Network (OPTN) of the Health Resources and Services Administration (HRSA) has developed information on Zika virus for organ transplant establishments and organ procurement organizations.

Jurisdictions should ensure procedures are in place with blood collection establishments for sharing information and coordinating response activities related to presumed viremic blood donors. Jurisdictions should also strengthen communication and information sharing procedures with local tissue collection establishments regarding Zika virus and tissue donations.

In addition to its critical role in protecting health, blood donation screening for Zika virus can enhance surveillance efforts and inform prevention and response measures. CDC efforts in these areas include the following:

- Providing consultation and guidance to help state, local, and tribal jurisdictions reduce the risk of transfusion- or tissue-related transmission (e.g., semen) of Zika virus.
- Establishing criteria for health departments to report blood donors with Zika infection to CDC's ArboNET.
- Working with state and local health officials to ensure that geographic areas with Zika virus transmission risk are posted on the CDC Zika virus website to assist blood collection and tissue recovery establishments in identifying areas requiring blood and tissue safety intervention (see *Communicating Geographic Areas with Zika Virus Transmission Risk* section).
- Providing guidance and technical assistance, as needed, to state or local jurisdictions and blood collection and tissue recovery establishments in following up with positive donors, reporting of donors

with Zika virus infection to ArboNET, and investigating suspected cases of transfusion- and transplant-transmitted infections.

Communication

When preparing for local Zika virus transmission, jurisdictions should follow risk communication principles to immediately communicate and effectively address concerns about Zika.

- Maintain credibility and public trust by regularly providing timely, accurate, and actionable information about what is known and unknown about Zika virus and dispelling rumors and misinformation.
- Increase access to accurate information about Zika among affected populations (i.e., pregnant women and community members) and convey appropriate action messages for each audience.
- Ensure communication is sensitive to diverse cultural health beliefs and practices, preferred languages, health literacy, and other communication needs (for more information, consult the National Standards for Culturally and Linguistically Appropriate Services in Health and Health Care [CLAS]).
- Ensure communication messages are accessible to non-English speaking audiences.

Jurisdictions should ensure that communication activities achieve the following:

- Increase knowledge of vector control activities in affected communities.
- Increase the capacity of healthcare providers to share accurate health information about Zika prevention with pregnant women and women of reproductive age, their partners, and affected populations. This will likely require targeted outreach to healthcare providers caring for pregnant women and infants.
- Motivate action by community leaders and organizations (e.g., MotherToBaby and March of Dimes) to protect pregnant women and other people at risk, especially vulnerable populations, from Zika virus infection.
- Communicate how Zika is spread and how people can protect themselves.
- Distribute communication materials (i.e., fact sheets, web updates, video messages, press releases) explaining public health activities by local, state and CDC officials, including provider tools, responsive vector control activities and travel guidance.

CDC's communication activities in response to Zika virus include the following:

- Coordination with relevant stakeholders
    - Coordinate public announcements with local authorities and other agencies.
    - Coordinate with state/local press release or press conference to issue a CDC press statement or hold a press briefing with CDC leadership or subject matter experts, as appropriate.
    - Before press events, distribute key information to agencies, officials, and public health partners.
        - Partner organizations, including national and local chapters as applicable: American College of Obstetricians and Gynecologists, Association of State and Territorial Health Officers, Council of State and Territorial Epidemiologists, National Association of City

and County Health Officers, Pan American Health Organization, Society for Maternal-Fetal Medicine, and the World Health Organization
    - Federal partners: Assistant Secretary for Preparedness and Response, Centers for Medicare and Medicaid Services, Environmental Protection Agency, Food and Drug Administration, Health Resources and Services Administration, and Indian Health Service
    - Congressional staff and elected officials at multiple levels
- Communication of messages
  - As appropriate, issue press release/media statement(s) and support local and state Public Information Officers.
  - Convey health messages and resources to professionals (i.e., elinicians, health departments, and laboratories) and the public.
  - Communicate how Zika is spread and how people can protect themselves.
- Ensuring effectiveness of communication activities
  - Support state and local responders in adapting and tailoring CDC-produced information products designed to ensure consistency and clarity of messages regarding Zika, vector control activities, and clinical guidance.
  - Monitor and assess news media, social media, and public inquiries to update or correct information delivered as needed.
  - Engage with relevant target audiences regularly to update and improve messaging and uptake.

Response

In the event of suspected or confirmed local transmission, state health officials should notify designated officials and the CDC Emergency Operation Center at 770-488-7100 or eocreport@cdc.gov.

CDC will work closely with the state health department to balance consistency in Zika virus response activities nationally with specific requirements of individual states and localities. CDC will provide support and assistance as needed in confirming cases, determining appropriate geographic areas for interventions, rapidly conducting an epidemiologic investigation, and enhancing surveillance activities, entomologic evaluation, and risk communication. CDC can provide on-the-ground assistance via a CDC Emergency Response Team, as described below. Although blood donation screening has been implemented in all US states and territories, CDC will continue to post information about geographic areas with Zika virus transmission risk on a designated section of the CDC Zika virus website to assist in identifying areas with risk of Zika for the purposes of blood and tissue safety intervention.

CDC continually reviews data and issues clinical guidance and testing recommendations focused on women of reproductive age, pregnant women, and infants. Pregnant women and women planning a pregnancy, as well as infants born to women infected with Zika virus during pregnancy, are a priority. For women and couples living in areas with risk of Zika who wish to delay or prevent pregnancy, CDC recommends that healthcare providers discuss how to prevent unintended pregnancy and offer the full range of FDA-approved contraceptive methods.

In all stages of the response, CDC will provide educational materials and targeted messages designed to reach pregnant women, men and women of reproductive age, and their healthcare providers.

CDC Emergency Response Teams (CERT)

When a suspected or confirmed case of local transmission is identified, CDC will work with the state or tribal health authorities to determine if CERT support is needed. CERT(s) may be requested by state, local, or tribal health authorities through the CDC Emergency Operations Center at 770-488-7100 or eocreport@cdc.gov. CDC will review the request and, if approved, will coordinate the mission and logistics of the CERT deployment with the health or emergency management authorities.

The composition of the CERT will depend on the needs of the state/local or tribal health authorities and will be a joint decision of the state/local or tribal health authority and CDC. CERTs can provide on-the-ground technical assistance in epidemiology, vector control, pregnancy and birth defects, blood and tissue safety, and risk communication, as well as community engagement, response management, and logistics. The team's resources include experts who specialize in detecting and controlling mosquito-borne diseases, case-investigation/ascertainment and surveillance, identifying and studying insects and vector control, and laboratory diagnostics for responding to the challenges presented by Zika. Specifically, CERTs can

- Assist with epidemiologic investigation of known cases to determine the timing and source of infection (travel-related, sexual, mosquito-borne, or other) through interviews with suspect cases, family, and possibly primary care providers.
- Assist with clinical outreach to healthcare providers caring for pregnant women and infants.
- Assist with clinical laboratory reporting to healthcare providers and laboratory interpretation.
- Assist with collection of data for the pregnancy registries and birth defects surveillance.
- Provide technical assistance and education on the clinical management of pregnant women and infants affected by Zika to state, local, and tribal health officials and providers.
- Work with existing local vector control programs to fill gaps around implementing local vector control measures
- Enhance or implement mosquito surveillance (if absent) to determine the type, distribution, and population size of competent *Aedes* mosquito species.
- Support community engagement efforts to implement vector control strategies and programs.
- Provide communication research, media and technical assistance, and audience-focused materials to help local health departments institute risk communication campaigns to provide information about the risk of Zika virus infection and personal measures people can take to decrease their risk for infection and adverse outcomes, with a focus on protecting pregnant women.
- Facilitate outreach to the local medical community to test and report suspect cases and to provide clear and actionable prevention information to patients, including prevention of both mosquito-borne and sexual transmission.
- Support staffing needs (as CDC resources permit) for state, local, or tribal health departments to enhance surveillance for Zika virus infection in people.

- Provide on-site training or assistance in performing laboratory tests for Zika infection, including scale up of local laboratory capacity or rapid transport of specimens to reference laboratories.

Communicating Geographic Areas with Zika Virus Transmission Risk

In the event that Zika virus transmission occurs at an intensity that presents a risk to pregnant women, CDC in consultation with states will issue domestic travel guidance for pregnant women to avoid or consider postponing travel to the affected area, as well as prevention, laboratory testing, and preconception counseling guidance. To keep the public informed, CDC will provide travel information and trip planning recommendations to the public and indicate areas of Zika transmission risk with guidance, maps, and case counts. CDC will also assist blood collection and tissue recovery establishments in identifying areas requiring blood and tissue safety intervention by posting this information on a designated website.

CDC has identified two types of geographic areas to describe where Zika virus-related domestic travel, testing, and other guidance applies: Zika cautionary areas (designated as yellow on map) and Zika active transmission areas (designated as red on map) (Appendix A). The designation of these areas can be revised or removed when public health assessment suggests a change in risk in consultation with CDC and state and local officials.

Surveillance and public health interventions implemented in and around these areas should be determined based on risk assessments for further local transmission (boundaries may vary by intervention). Risk assessments should include factors such as history of previous local dengue or chikungunya virus transmission; population density; large numbers of the mosquitoes that spread Zika; locations of recent travel-associated cases; local travel patterns (i.e., areas known to have a high number of travelers to affected areas, areas with previously identified cases of travel-associated dengue and chikungunya); and other risk factors (e.g., lack of air conditioning or screens).

Surveillance and response activities should be scaled based on the intensity and geographic extent of transmission. CDC can provide consultation and CERT assistance with scaling up surveillance and response activities, as needed.

Zika active transmission areas (red areas)

A Zika active transmission (red) area is a geographic area in which local, state, and CDC officials have identified the presence of confirmed, multiperson local mosquito-borne transmission and have determined that the intensity of Zika virus transmission presents a significant risk of Zika virus infection, posing a risk to pregnant women and blood and tissue safety. In a red area, a combination of preventive interventions should be implemented, most importantly travel guidance recommending pregnant women not travel to the area. Blood collection and tissue recovery establishments should refer to FDA guidance for detailed recommendations (see *Blood and Tissue Safety* section). Testing, prevention, and preconception counseling recommendations for red areas can be found here.

When defining a red area, states in consultation with CDC, should designate the smallest, easily identifiable location, with a minimum of 1-mile diameter that completely encompasses the geographic area of significant risk, particularly to pregnant women, as delineated by epidemiologic, entomologic, and environmental investigation. The boundaries of this area should be communicated to the public using terminology and landmarks recognizable to residents and visitors, such as street-level borders, a neighborhood, a zip code area, a city, or a county. After a period of 45 days with no additional confirmed local transmission cases and no suspected local transmission cases under active investigation, a red Zika active transmission area should be designated as a cautionary (yellow) area, as described below.

Zika cautionary areas (yellow areas)

A Zika cautionary (yellow) area is a geographic area in which local mosquito-borne transmission has been identified and pregnant women and blood and tissue safety are at some undetermined risk, but evidence is lacking on whether the intensity of transmission is widespread and sustained. Pregnant women should consider postponing travel to yellow areas. Blood collection and tissue recovery establishments should refer to FDA guidance for detailed recommendations (see *Blood and Tissue Safety* section). Testing, prevention, and preconception counseling recommendations for yellow areas can be found here.

Acknowledging the need to be adaptable and responsive to local circumstances, a yellow area may be established in one of two ways: (1) as a cautionary area surrounding a Zika active transmission (red) area, or (2) as a cautionary (yellow) area alone. When a red area is established, a yellow area is implemented simultaneously around it, with the yellow area boundaries defined by the borders of the county, city, or another similar jurisdiction with easily identifiable borders for public communication. Removal or revision of the yellow area may be considered when public health assessment indicates a clear change in risk (e.g., a period of 45 days after the red area designation ends, with no additional confirmed local transmission cases, no suspected local transmission cases under active investigation and enhanced surveillance in place).

If a red area has not been defined, a yellow area may be designated if there are three or more local transmission cases without an epidemiologic link (e.g., non-household cases) within a 5-mile diameter over a 45-day period. Preferably, case locations should be mapped by the location of the most likely exposure or if necessary, by home or neighborhood residence. Similar to a yellow area surrounding a red area described above, the boundaries of a "stand-alone" yellow area are defined by the borders of the county, city, or another similar jurisdiction with easily identifiable borders for public communication. Removal or revision of the yellow area may be considered when public health assessment indicates a clear change in risk (e.g., a period of 45 days after the yellow area is implemented, with no additional confirmed local transmission cases and no suspected local transmission cases under active investigation and enhanced surveillance in place). Additional reporting factors to consider before removal or revision of the yellow area, especially in jurisdictions balancing multiple competing priorities, include timeliness of case investigations, laboratory testing, and delays in data sharing.

Response to a Suspect Case of Local Mosquito-borne Transmission

In response to a suspect case of local mosquito-borne transmission, state and loeal health authorities should

- Initiate an epidemiologic investigation to determine the timing and potential source of infection (i.e., locations of possible mosquito exposure, travel within CONUS) (see Possible Local Mosquito-borne Transmission Zika Virus Case Investigation Form).
- Be prepared to share vital information and coordinate surveillance and vector control efforts with vector control officials.
- Implement local vector surveillance and control, as appropriate.
- Communicate with clinicians caring for pregnant women and infants about the risks of Zika and disseminate CDC guidance for these populations.
- Ensure that state and local maternal and child health and birth defects programs are integrated into Zika virus planning and response activities.
- If applicable, coordinate with blood collection establishments to begin traceback and other follow-up activities related to presumptive viremic blood donors.
- Verify procedures and points of contact with local tissue collection establishments regarding Zika virus and tissue donations.

Response to a Confirmed Case of Local Mosquito-borne Transmission

Local transmission by mosquitoes should be assumed whenever a case is confirmed and other routes of exposure (e.g., travel, sexual contact, transfusion) have been evaluated and likely ruled out. Under these circumstances, state, local, and tribal jurisdictions should implement surveillance for Zika virus disease around the home of the confirmed, locally acquired case and any other likely locations of exposure identified through the case investigation. The principal objectives of this surveillance should be to define the frequency and geographic extent of local transmission. Tools to assist in conducting epidemiologic investigations and surveillance in households, workplaces, and the community can be found here.

Because dengue and chikungunya virus infections share a similar geographic distribution with anticipated Zika virus distribution and acute symptoms of infection with all three viruses are similar, patients under investigation for Zika virus infection should also be evaluated and managed for possible dengue or chikungunya virus infection. It is important to identify dengue virus infections because proper clinical management of dengue can improve outcomes for patients.

In response to a confirmed case of local mosquito-borne transmission, state and local health authorities should

- Notify CDC of the investigation and provide basic epidemiologic information regarding the confirmed case to ensure coordination of efforts between the jurisdiction and CDC. (see Zika Line List Template)

- Determine if CERT support is needed.
- Identify the physical location of the case patient's most likely place(s) of exposure (e.g., home, work, other US location, if recent travel). (see Possible Local Mosquito-borne Transmission Zika Virus Case Investigation Form)
- Implement targeted surveillance activity around suspected area(s) of local transmission to identify if other recent cases are from same/nearby mosquito pool; these activities can help quickly confirm local transmission. (see sample forms in Toolkit for Investigating Possible Local Mosquito-Borne Transmission of Zika Virus)
  - For household members:
    - Assess for symptoms of Zika virus disease (e.g., within 8 weeks prior to the case patient's symptom onset).
    - Evaluate relationship to case patient, pregnancy status and plans to become pregnant, if applicable.
    - Collect urine and serum to test for recent Zika virus infection.
    - Assess travel and other potential exposures, if applicable.
    - Ask about history of blood or tissue donation.
  - For close neighbors/neighborhood in suspected area/workplace with outdoor exposure
    - Conduct house-to-house survey of any available people, or survey at local gathering place or workplace, to identify any recently symptomatic people (e.g., within 8 weeks prior to the case patient's symptom onset)
    - If symptomatic, obtain specimens to test for recent Zika virus infection.
- Conduct enhanced surveillance in areas contiguous to the location where local transmission likely occurred, especially those with documented vector activity and high travel volume to the affected area.
  - Determine if additional identified suspect cases are likely to represent a single transmission chain or separate occurrences.
  - Ensure adequate surveillance of pregnant women and infants, including testing when indicated.
- In coordination with CDC, evaluate the need to define a Zika cautionary (yellow) area. If a yellow area is established:
  - Communicate travel, testing, and related guidance associated with cautionary areas to target audiences and partners, including the boundaries of such areas.
  - Communicate the location(s) of any such area(s) to blood collection and tissue recovery establishments.
- In coordination with vector control officials, implement local vector surveillance and control as appropriate.
  - Intensify vector surveillance and resistance testing efforts in identified geographic area(s).
  - Consider focal or area-wide treatments with larvicides and adulticides using application methods (truck or aerial) appropriate for the treatment area(s).
  - Intensify source reduction efforts.
  - Consider adding community-based adult mosquito control consisting of outdoor residual spraying and space spraying if necessary.

- Consider targeted indoor residual spraying in areas where air conditioning and screens are not widely available.
- Increase coordination of response activities with state and local maternal and child health and birth defects programs.
- Communicate with blood collection and tissue recovery establishments (see *Blood and Tissue Safety* section) to identify additional cases.
- If applicable, coordinate with blood collection establishments to begin traceback and other follow-up activities related to presumptive viremic blood donors.
- Further expand laboratory testing for symptomatic people, including pregnant women, and assess laboratory surge capacity for anticipated increased testing volume.
- Augment clinician outreach, education, and communication activities to healthcare providers in the county or jurisdiction through existing local channels (e.g., messages through local medical societies and local chapters of ACOG/AAP, Health Alert Network messages [HANs], conference calls).
  - Communicate with clinicians caring for pregnant women and infants about the risks of Zika and disseminate CDC guidance.
  - Emphasize the importance of testing pregnant women and infants.
- Prepare and issue a media statement in coordination with CDC and involved local health departments.
- Hold press conferences/events about confirmed local transmission Zika case, ongoing investigations, and updates. Pre-release copies of the press release and Q&As to
  - State and local health departments
  - Responding health department unit, environmental health unit, law enforcement, and local elected officials
- Conduct appropriate risk communication, following established principles (e.g., be first, be right, be credible).
  - Inform the public about what is known and what is not known.
  - Provide actions people can take to protect themselves and their families to reduce the risk of infection through mosquito bites and sexual transmission, and minimize the potential for public misunderstanding, rumors, and fear.
  - Use available communication channels appropriate for the local community.
  - Engage with pregnant women, women of reproductive age, and their families/partners with personal protective measures recommendations (e.g., steps to prevent mosquito bites and sexual transmission)
- Provide information to pregnant women and women of reproductive age about the presence of Zika virus in the local area and what precautions they should take to prevent being infected with or avoid Zika virus exposure during pregnancy. Information should also be made available for their sexual partners.
- Implement community outreach efforts to encourage people with clinically compatible illnesses to seek care (and testing for confirmation, when appropriate).
- Monitor local news stories and social media posts to determine if information is accurate, identify messaging gaps, and make adjustments to communication materials, as needed.

Response to Confirmed, Multiperson Local Mosquito-borne Transmission

In response to confirmed, multiperson local mosquito-borne transmission, state and local health authorities should

- Determine if CERT support is needed.
- In coordination with CDC, define the boundaries of the Zika active transmission (red) area and the surrounding Zika cautionary (yellow) area to communicate geographic areas with Zika virus transmission risk.
  - Communicate travel, testing, and related guidance associated with these areas to target audiences and partners, including the boundaries of such areas.
  - Communicate the location(s) of any such area(s) to blood collection and tissue recovery establishments.
- Prepare and issue a media statement in coordination with CDC and involved local health departments.
- Continue to conduct enhanced surveillance activities to identify additional cases of local transmission.
- Continue vector surveillance and control measures as guided by an entomologic evaluation of the area.
  - Consider intensifying and expanding area-wide treatments with larvicides and adulticides using application methods (truck or aerial) appropriate for the scale of the treatment area.
  - Conduct intensive source reduction in affected area(s).
  - Consider intensifying targeted indoor residual spraying to vulnerable homes if air conditioning and screens are not widely available.
- Continue to enhance coordination of response activities with state and local maternal and child health and birth defects programs.
- Further escalate clinician outreach and communication activities to healthcare providers in the county or jurisdiction through existing local channels (e.g., messages through local medical societies and local chapters of ACOG/AAP/AMCHP, Health Alert Network messages [HANs], conference calls).
  - Intensify communication with clinicians caring for pregnant women and infants about the risks of Zika and disseminate CDC guidance.
- Recommend testing to all pregnant women (symptomatic and asymptomatic) and other people who have symptoms who live in or travel to a red area and the surrounding yellow area.
  - Implement laboratory surge plans to ensure timely testing.
  - Prioritize pregnant women for diagnostic testing, followed by symptomatic people who are not pregnant, except in circumstances where testing a limited number of symptomatic people is crucial for monitoring key epidemiologic factors (e.g., changes in transmission intensity or extent).
  - Provide guidance to laboratories as needed.
- Communicate with blood collection and tissue recovery establishments (see *Blood and Tissue Safety* section) to identify additional cases.

- If applicable, coordinate with blood collection establishments to begin traceback and other follow-up activities related to presumptive viremic blood donors.
- With CDC assistance, conduct risk communication activities that ensure information and prevention recommendations reach intended audiences within their jurisdictions. Communication activities should
    - Describe the area where Zika virus transmission is thought to be occurring based on the best available epidemiologic, entomologic, and environmental information.
    - Identify estimated date when local Zika virus transmission began.
    - Describe the surveillance and response efforts taking place in the affected area and provide objective assessments of the situation and scale of the public health threat.
    - Communicate the importance and availability of testing to all pregnant women (symptomatic and asymptomatic) and other people who have symptoms who live in or travel to red area and the surrounding yellow area.
    - Advise healthcare providers of pregnant women of the appropriate steps for Zika virus testing in accordance with CDC guidance.
    - Reinforce recommendations for pregnant women and women wishing to conceive and their sex partners.
    - Provide advice about ways to reduce mosquito populations around the home.
    - Advise pregnant women and their sex partners to take steps to prevent mosquito bites and sexual transmission (e.g., wearing insect repellent and using condoms or not having sex to protect themselves from Zika virus infection).
    - Identify and provide resources (e.g., insect repellent, window screens, condoms) for specific communities as necessary to minimize exposure risk, particularly for pregnant women and their partners.
    - Provide guidance to schools, such as the Interim Guidance for School District and School Administrators in the Continental United States and Hawaii.
    - Engage early with businesses, including blood collection and tissue recovery establishments and labor stakeholders, to prepare for the potential short- and long-term economic effects.
    - The Occupational Safety and Health Administration (OSHA) and the National Institute for Occupational Safety and Health (NIOSH) have published interim guidance for protecting workers from occupational exposure to Zika virus
- Implement expanded state and local intervention plans for all vulnerable populations, specifically pregnant women, women at risk for unintended pregnancy, women and men planning pregnancy, and children. Recommendations for reducing risk should target everyone, but particularly pregnant women and women at risk for unintended pregnancy who live in, work in, or must travel to an area with risk of Zika.
- Identify statewide resources for caring for infants and children with Zika-associated birth defects, developmental concerns, and other related outcomes.
- Encourage providers to join an American Academy of Pediatrics/American College of Obstetricians and Gynecologists (AAP/ACOG) provider network (when established).
- Report all cases to ArboNET, using the Council of State and Territorial Epidemiologists (CSTE) approved case definitions for non-congenital and congenital Zika virus infection and disease.

- Report all pregnant women with laboratory evidence of possible Zika virus infection and their infants to the US Zika Pregnancy Registry (USZPR) for monitoring and follow up on pregnancy and infant outcomes.
- Work with the state-based birth defects surveillance system to report all infants with birth defects potentially related to Zika virus to Zika Active Birth Defects Surveillance at CDC.
- Continue to monitor the status of local transmission on a weekly basis, at a minimum. The geographic area(s) for Zika virus intervention should be adjusted based on current information.
    - Environmental conditions not conducive to mosquito activity, or other evidence that indicates the risk of Zika virus transmission has been reduced, should also be considered when scaling down interventions.
    - Implement a protocol and communication strategy when interventions are changed or rightsized.

Future Zika Virus Preparedness

As jurisdictions continue to address the threat posed by Zika virus, it is vital to build on the plans and capacities established over the past year by incorporating evolving knowledge of Zika virus and the methods used to combat its spread. CDC will assist jurisdictions in protecting their residents by improving surveillance, enhancing vector control, facilitating appropriate testing, and providing messaging to clinicians and the public.

The full range of health effects caused by Zika virus is currently unknown. However, if a pregnant woman is infected, the virus can pass to her fetus during pregnancy and cause congenital Zika syndrome. Congenital Zika syndrome is a pattern of birth defects associated with Zika virus infection during pregnancy that includes brain abnormalities, eye abnormalities, and hearing loss. Research continues to further define the spectrum of anomalies associated with Zika virus infection during pregnancy.

Zika virus poses a serious risk to public health; therefore, it is essential that jurisdictions remain engaged in preparedness and response activities. States, locals, and tribes should use the guidance provided in this document and referenced throughout to prepare for and respond to the threat of Zika virus in their jurisdictions.

Appendix A - Domestic Travel and Testing Guidance for Local Mosquito-borne Transmission of Zika Virus*

<table>
<tr><th>Risk Designation</th><th>Trigger On</th><th>Geographic Area with Transmission Risk</th><th>Trigger Off</th><th>Travel Guidance</th><th>Testing Guidance</th></tr>
<tr><td rowspan="2">Yellow Area</td><td>Confirmed local transmission† for ≥ three cases without an epidemiologic link within a 5-mile diameter over a 45-day period.</td><td>County, city, or other similar jurisdiction with easily identifiable borders for public communication.</td><td>Consider removing if there are no new cases of confirmed local transmission[§] in at least a 45-day period after the yellow area is implemented[¶].</td><td rowspan="2">Pregnant women should consider postponing travel to the geographic area.</td><td rowspan="2">All pregnant women who lived in, traveled to, or had sex without a condom with someone who lived in or traveled to area should be tested for Zika virus.</td></tr>
<tr><td>Implemented simultaneously with red area.</td><td>County, city, or other similar jurisdiction with easily identifiable borders for public communication.</td><td>Consider removing if there are no new cases of confirmed local transmission[§] for a period of 45 days after red area ends[¶].</td></tr>
<tr><td>Red Area</td><td>Confirmed multi-person transmission[**].</td><td>The smallest, easily identifiable location that completely encompasses the area at risk. Minimal area is 1-mile diameter.</td><td>No new cases of confirmed local transmission[§] identified for a period of 45 days[††].</td><td>Pregnant women should not travel to the geographic area.</td><td>All pregnant women who lived in, traveled to, or had sex without a condom with someone who lived in or traveled to area should be tested for Zika virus.</td></tr>
</table>

* Recommendations for pregnant women and other people that live in areas with local mosquito-borne transmission can be found here: https://www.cdc.gov/zika/geo/domestic-guidance.html. Additional guidance can also be found at https://wwwnc.cdc.gov/travel/page/us-citizens-living-in-areas-with-zika.

† A person who does not have risk factors for Zika virus acquisition through travel, sexual contact, or other known exposure with body fluids, and who tests positive for Zika virus infection per CDC laboratory guidance; **OR**

A blood donor identified through Zika virus screening of blood donations, who does not have risk factors for Zika acquisition through travel, sexual contact, or other body fluid exposure, and who has a positive Zika virus nucleic acid test (NAT) on screening AND confirmation through an approved confirmatory test algorithm.

§ And no suspect local transmission cases under investigation with enhanced surveillance in place.

¶ CDC and state/local public health officials should discuss likelihood of ongoing risk before removal of the yellow area designation.

** Three or more cases of confirmed local transmission in non-household members, with at least two cases with onsets greater than two weeks apart (the approximate survival of an infected mosquito), and less than 45 days in an approximate 1-mile diameter area.

†† After 45 days without a confirmed case of local transmission red area, CDC and state/lncal public health officials should discuss converting it to a yellow area.

For more information, please contact 1-800-CDC-INFO (232-4636)
TTY: 1-888-232-6348
www.cdc.gov
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Chikungunya Virus
Introduction in the Americas

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PREFACE

Chikungunya fever (CHIK) is an emerging, mosquito-borne disease caused by an *alphavirus*, Chikungunya virus (CHIKV). The disease is transmitted predominantly by *Aedes aegypti* and *Ae. albopictus* mosquitoes, the same species involved in the transmission of dengue.

Traditionally, CHIKV epidemics have shown cyclical trends, with inter-epidemic periods ranging from 4 to 30 years. Since 2004, CHIKV has expanded its geographical range, causing sustained epidemics of unprecedented magnitude in Asia and Africa. Although areas in Asia and Africa are considered to be endemic for the disease, the virus produced outbreaks in many new territories in the Indian Ocean islands and in Italy. This recent reemergence of CHIKV has heightened the world's public health awareness and concern about this virus.

Controlling the spread of arthropod-borne viruses (arboviruses) in the Americas has not been very successful. Dengue continues to ravage many areas in the Americas, reaching as far north as the United States and as far south as Argentina. During the first trimester of 2010, several dengue virus outbreaks in the Region occurred at unprecedented rates for this time of the year, especially in Central America and the Caribbean.

West Nile virus, another arbovirus recently introduced to the Americas, is now endemic in the Region. Over the last decade, West Nile virus has evolved epidemiologically and has expanded its geographic range in the Region from Canada to Argentina; in 2007, human and equine cases were detected in Argentina. Moreover, in 2010, three laboratory-confirmed cases of a related

arbovirus, the Saint Louis encephalitis virus, were reported in children from 6 to 8 years old in Argentina (the city of Buenos Aires and the province of Buenos Aires).

Although indigenous transmission of CHIKV does not occur in the Americas now, the risk for its introduction into local vector mosquito populations is likely higher than had previously been thought, especially in tropical and subtropical areas where *Ae. aegypti*, one of the main vectors of CHIKV, has a broad distribution. The broad distribution of competent vectors, coupled with the lack of exposure to CHIKV of the human population in the Americas, places this Region at risk for the introduction and spread of the virus. The resulting large outbreaks would likely tax existing health-care systems and the public health infrastructure, and could potentially cripple some of society's functioning.

Between 2006 and 2010, 106 laboratory-confirmed or probable cases of CHIKV were detected among travelers returning to the United States, compared to only 3 cases reported between 1995 and 2005. There also have been nine imported CHIK cases reported in the French territories in the Americas since 2006-three in Martinique, three in Guadeloupe, and three in Guyana. To date, none of the travel-related cases have led to local transmission, but these cases document an ongoing risk for the introduction and possible sustained transmission of CHIKV in the Americas.

There is no specific treatment for CHIKV infection, nor any commercially available vaccine to prevent it. Pending the development of a new vaccine, the only effective means of prevention are to protect individuals against mosquito bites. It should be noted, however, that the only available method for preventing an ongoing transmission of a possible CHIKV epidemic, namely the control of its vectors, has rarely been achieved and never has been sustained.

Given these factors, the Pan American Health Organizarion (PAHO), with the support of the Division of Vector-borne Diseases of the United States Centers for Disease Control and Prevention (DVBD, CDC), created a working group and convened a meeting in Lima, Peru, on July 21–23, 2010, to discuss the threat this virus represents and to examine measures that might be taken to mitigate this

threat (see Appendix I for additional details on the meeting). These preparedness guidelines are the result of this collaboration.

These guidelines are intended to be adapted by each Member Country. They are designed to increase awareness about the threat and to provide the necessary tools to put in place the best possible strategies to prevent the importation of CHIKV into the Region, or to control it if introduced. These guidelines provide guidance on how to detect an outbreak of the disease, conduct pertinent epidemiological investigations, and prevent or mitigate the spread of the disease throughout the Region.

We encourage everyone working to apply these guidelines to take into account all the knowledge available and their own country's capability to cope with the introduction of CHIKV. Steps should be taken now to put in place the necessary measures that will decrease the impact that this new arbovirus could have in our Region.

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ABBREVIATIONS AND ACRONYMS

Ae.	*Aedes*
BSL-3	Biosafety level 3
CAREC	Caribbean Epidemiology Centre
CBC	Complete blood count
CDC	United States Centers for Disease Control and Prevention
CHIK	Chikungunya fever
CHIKV	Chikungunya virus
CIRE	Les Cellules de l'Institut de veille sanitaire en région
CPE	Cytopathic effects
CSF	Cerebrospinal fluid
Ct	Cutoff
DEET	N,N-Diethyl-meta-toluamide
DVBD	Division of Vector-borne Diseases
EEE	Eastern equine encephalitis
ELISA	Enzyme-linked immunosorbent assay
FAQ	Frequently asked question
GIS	Geographic Information System
HHS	U. S. Department of Health and Human Services
HI	Hemaglutination Inhibition
HSD/IR/D	Area of Health Surveillance and Disease Prevention and Control / Alert and Response and Epidemic Diseases / Program for Dengue
HSD/IR/V	Area of Health Surveillance and Disease Prevention and Control / Alert and Response and Epidemic Diseases / Program for Viral Diseases
IFA	Immunofluorescence assay
IgG	Immunoglobulin G

IgM	Immunoglobulin M
IHR	International Health Regulations
IRS	Indoor residual sprays
IT	Insecticide-treated
IVM	Integrated vector management
JE	Japanese encephalitis
JIC	Joint Information Center
MAC-ELISA	IgM antibody capture enzyme-linked immunosorbent assay
NAT	Nucleic acid amplification testing
NGO	Non-Governmental Organization
NMRC	United States Naval Medical Research Center
NMRCD	United States Naval Medical Research Center Detachment
NSAID	Non-steroidal anti-inflammatory agent
PAHO	Pan American Health Organization
PCR	Polymerase chain reaction
Pfu	Plaque-forming unit
PHEIC	Public Health Emergency of International Concern
PIO	Public Information Officer
PRNT	Plaque reducion neutralization test
PWR/PAN	Pan American Health Organization, World Health Organization Representative/Panama
RELDA	Red de laboratorios de dengue de las Américas
RNA	Ribonucleic acid
RT-PCR	Reverse transcriptase-polymerase chain reaction
SFV	Semliki Forest virus
SLE	St. Louis encephalitis
SMS	Short Message Service
TMB	Tetramethylbanzidine base
THAN	Travel health alert notice
VEE	Venezuelan equine encephalitis
WEE	Western equine encephalitis
WHO	World Health Organization
WHOCC	World Health Organization Collaborating Centre
WHOPES	World Health Organization Pesticide Evaluation Scheme

1. BACKGROUND AND RATIONALE

Since 2004, Chikungunya virus (CHIKV) has been causing large epidemics of chikungunya fever (CHIK), with considerable morbidity and suffering. The epidemics have crossed international borders and seas, and the virus has been introduced into at least 19 countries by travelers returning from affected areas. Because the virus has been introduced into geographic locations where the appropriate vectors are endemic, the disease could establish itself in new areas of Europe and the Americas. The possibility that CHIKV could become established in the Americas has heightened awareness of the need to develop guidelines for the prevention and control of CHIK in PAHO's Member Countries. This document is meant to serve as a guideline that individual countries can use as the basis for their CHIKV surveillance, prevention, and control programs.

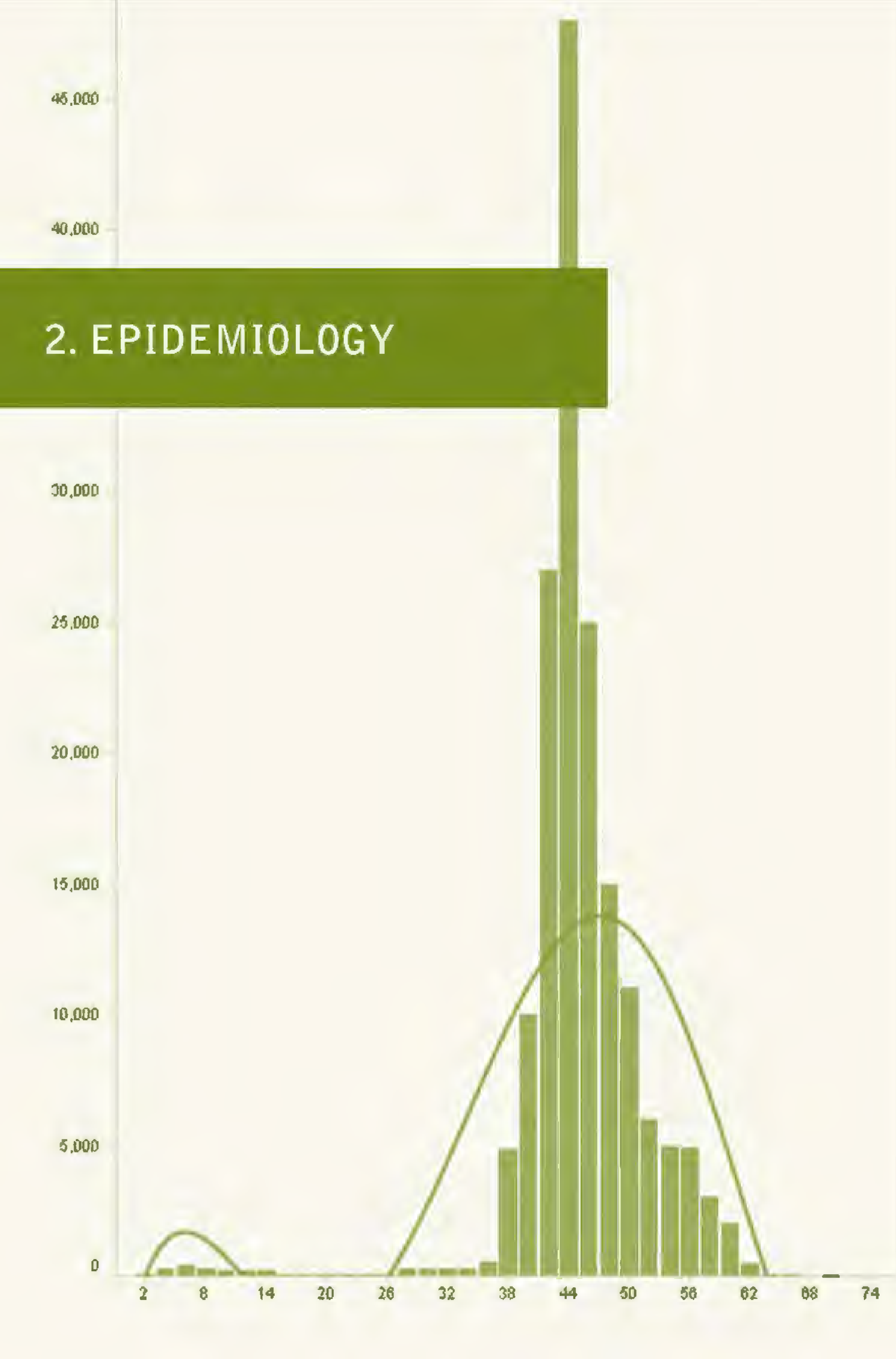
TARGET OF THESE GUIDELINES:

These guidelines are intended to be used by health workers, public health officials, and vector control specialists at the national, district, and sub-district levels.

OBJECTIVES:

General objectives are the prevention, detection, and timely response to outbreaks of CHIK through surveillance, case detection, investigation, and the launching of public health actions.

2. EPIDEMIOLOGY



CHIKV is an RNA virus that belongs to the *Alphavirus* genus in the family *Togaviridae*. The name *chikungunya* derives from a word in Makonde, the language spoken by the Makonde ethnic group living in southeast Tanzania and northern Mozambique. It roughly means "that which bends," describing the stooped appearance of persons suffering with the characteristic painful arthralgia.

Epidemics of fever, rash, and arthritis resembling CHIK were reported as early as the 1770s. However, the virus was not isolated from human serum and mosquitoes until an epidemic in Tanzania in 1952–1953.[1] Subsequent outbreaks occurred in Africa and Asia, many of them affecting small or rural communities. In Asia, however, CHIKV strains were isolated during large urban outbreaks in Bangkok, Thailand, in the 1960s and in Calcutta and Vellore, India, during the 1960s and 1970s.[2,3]

Recent Outbreaks

After the initial identification of CHIKV, sporadic outbreaks continued to occur, but little activity was reported after the mid-1980s. In 2004, however, an outbreak originating on the coast of Kenya subsequently spread to Comoros, La Réunion, and several other Indian Ocean islands in the following two years. From the spring of 2004 to the summer of 2006, an estimated 500,000 cases had occurred.

The epidemic spread from the Indian Ocean islands to India, where large outbreaks occurred in 2006. Once introduced in India, CHIKV spread to 17 of the country's 28 states, infecting more than 1.39 million people before the end of the year. The outbreak in India continued into 2010, with new cases appearing in areas that had not been affected in the epidemic's early phase. Viremic travelers also spread outbreaks from India to the Andaman and Nicobar Islands, Sri Lanka, the Maldives, Singapore, Malaysia, Indonesia. Concern over the spread of CHIKV peaked in 2007, when the virus was found to be spreading autochthonously (human-to-mosquito-to-human) in northern Italy after being introduced by a viremic traveler returning from India.[4] The attack rates in communities that have been affected in the recent epidemics ranged from 38%–63%, and in many of these countries cases continue to be reported, albeit at reduced levels. In 2010, the virus continued to cause illness in India, Indonesia, Myanmar, Thailand, and the Maldives; it also has resurged in La Réunion. In 2010, imported cases also were identified in Taiwan, France, and the United States. These cases were infected viremic travelers returning from Indonesia, La Réunion, and India, respectively.

During the recent outbreaks, individuals viremic with CHIKV were found in the Caribbean (Martinique), the United States, and French Guiana.[5] All of them had returned from areas with endemic or epidemic CHIKV transmission; thus, these cases were not due to autochthonous transmission. All of these areas have competent mosquito vectors and naïve hosts, however, and thus could support endemic transmission of CHIKV in the Americas. Given these factors, CHIKV has the capacity to emerge, re-emerge, and quickly spread in novel areas, which makes heightened surveillance and preparedness a priority.

Transmission Dynamics

Vectors

There are two main vectors of CHIKV, *Aedes aegypti* and *Ae. albopictus*. Both mosquito species are widely distributed throughout the tropics with *Ae. albopictus* also present at more temperate latitudes. Given the vectors' distribution throughout the Americas, the entire Region is susceptible to the virus' invasion and spread.

Reservoirs

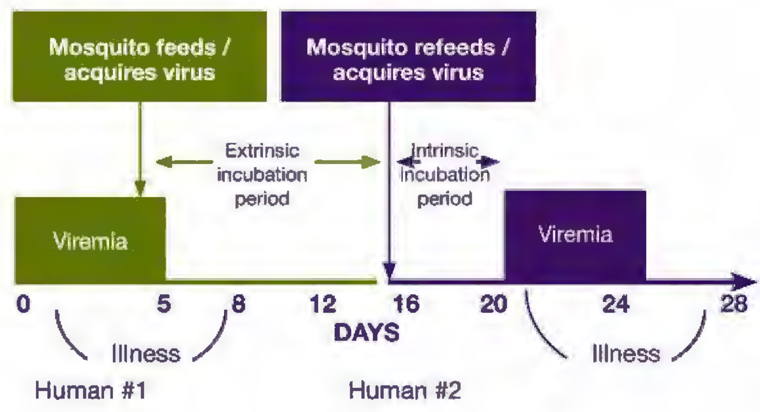
Humans serve as the primary CHIKV reservoir during epidemic periods. During inter-epidemic periods, several vertebrates have been implicated as potential reservoirs, including non-human primates, rodents, birds, and some small mammals.

Incubation Periods

Mosquitoes acquire the virus from a viremic host. Following an average extrinsic incubation of 10 days, the mosquito is then able to transmit the virus to a naïve host, such as a human. In humans bitten by an infected mosquito, disease symptoms typically occur after an average intrinsic incubation period of three-to-seven days (range: 1–12 days) (Figure 1).

EPIDEMIOLOGY

Figure 1. **Extrinsic and intrinsic incubation periods for Chikungunya virus.**



Susceptibility and Immunity

All individuals not previously infected with CHIKV (naïve individuals) are at risk of acquiring iufection and developiug disease. It is believed that once exposed to CHIKV, individuals will develop long lasting immunity that will protect them against reiufection.

EPIDEMIOLOGY

Summary of Epidemiology Section

- CHIKV is an RNA virus that belongs to the *Alphavirus* genus in the family *Togaviridae*.
- The attack rates in communities that have been affected in the recent epidemics ranged from 38%–63%.
- The two major vectors of CHIKV are *Ae. aegypti* and *Ae. albopictus*; both mosquitoes are widely distributed throughout the tropics and *Ae. albopictus* is present at more temperate latitudes.
- CHIK is not known to be circulating in the Americas; however, the risk of introduction is high due to travel importation, competent vectors (same vectors as dengue), and population susceptibility.

3. CLINICAL

3A. Clinical Presentation of Acute Disease

Following the bite of a mosquito infected with CHIKV, most individuals will present with symptomatic disease after an incubation period of three to seven days (range: 1–12 days). Not all individuals infected with the virus develop symptoms, however. Serosurveys indicate that between 3% and 28% of persons with antibodies to CHIKV have asymptomatic infections.[6–7] Individuals acutely infected with CHIKV, whether clinically apparent or asymptomatic, can contribute to the spread of the disease if the vectors that transmit the virus are present and active in the same location.

CHIKV can cause acute, subacute, and chronic disease. Acute disease is most often characterized by sudden onset of high fever (typically greater than 102°F [39°C]) and severe joint pain.[8–10] Other signs and symptoms may include headache, diffuse back pain, myalgias, nausea, vomiting, polyarthritis, rash, and conjunctivitis (Table 1).[11] The acute phase of CHIK lasts for 3–10 days.

CLINICAL

Table 1. **Frequency of acute symptoms of CHIKV Infection.[a]**

Symptom or sign	Frequency range (% of symptomatic patients)
Fever	76–100
Polyarthralgias	71–100
Headache	17–74
Myalgias	46–72
Back pain	34–50
Nausea	50–69
Vomiting	4–59
Rash	28–77
Polyarthritis	12–32
Conjunctivitis	3–56

[a]Table compiled from a number of different studies.[8, 9, 12-17]

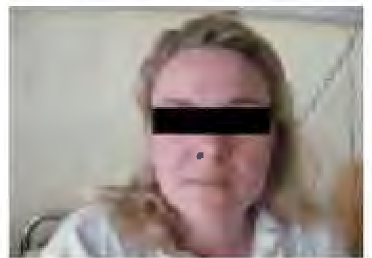
- Fever typically lasts from several days up to a week. The fever can be continuous or intermittent; a drop in temperature is not associated with worsening of symptoms, however. Occasionally, the fever may be associated with relative bradycardia.

- Joint symptoms are usually symmetric and occur most commonly in hands and feet, but they can affect more proximal joints. Swelling can also be seen and is often associated with tenosynovitis. Patients are often severely incapacitated due to pain, tenderness, swelling, and stiffness. Many patients cannot perform normal tasks or go to work, and many will be confined to bed due to these symptoms.

- Rash usually occurs two to five days after onset of fever in approximately half of all patients. It is typically maculopapular, involving the trunk and extremities, but can also include palms, soles, and face. The rash can also present as a diffuse erythema that blanches with pressure. In infants, vesiculobullous lesions are often the most common skin manifestation.

There are no significant pathognomonic hematologic findings seen with CHIKV infections. Abnormal laboratory findings can include mild thrombocytopenia (>100,000/mm^3), leukopenia, and elevated liver function tests. Erythrocyte sedimentation rate and C-reactive protein are usually elevated.

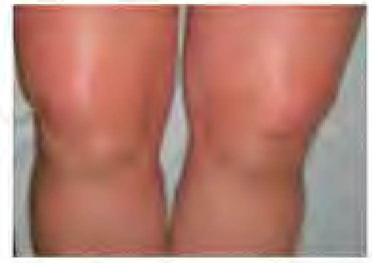
Rarely, severe forms of the disease can occur with atypical manifestations (see Section 3B). Fatalities related to CHIKV infection are thought to be uncommon. However, an increase in crude death rates was reported during the 2004–2008 epidemics in India and Mauritius.[18, 19]



A. Edematous rash of the face

B. Edematous poylarthritis of the hands

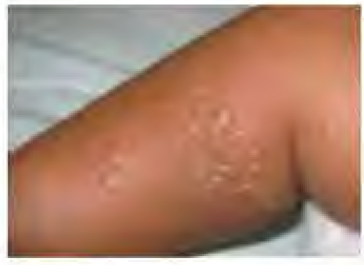
C. Erythema that blanches with pressure



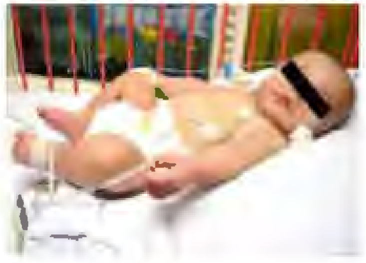
D. Periarticular swelling and joint effusion in knees

E. Maculopapular rash in trunk and extremities

F. Maculopapular rash in extremities, including palms



G. Bullous lesions in infant leg



H. Infant with maculo-papular rash, petechial spots and erythema of upper and lower limbs associated with edema of the extremities

I. End of the acute stage. Swollen hands and fine desquamation

J. Hyperpigmentation

K. Tenosynovitis in hands

L. Tenosynovitis in ankle

M. Elbow hygroma

N. Swollen and stiff hands in a 55-year-old man who was infected 5 years earlier

Credits:

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(B) Pr. Fabrice Simon. Department of Infectious Diseases and Tropical Medicine. Laveran Military Teaching Hospital. Marseille, France. **Previously published in:** Simon F. et al. Chikungunya infection: an emerging rheumatism among travelers returned from Indian Ocean islands. Report of 47 cases. Medicine (Baltimore). 2007 May ;86(3):123-37.

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(H) Dr. Stéphanie Robin and Dr. Duksha Ramful, Service de Pédiatrie, CHR Félix Guyon, Saint-Denis de La Réunion

3B. Atypical Manifestations

Although most CHIKV infections result in fever and arthralgias, atypical manifestations can occur (Table 2). These manifestations can be due to the direct effects of the virus, immunologic response to the virus, or drug toxicity.

Table 2. **Atypical manifestations of CHIKV infection.**

System	Clinical manifestations
Neurological	Meningoencephalitis, encephalopathy, seizures, Guillain-Barré syndrome, cerebellar syndrome, paresis, palsies, neuropathy
Ocular	Optic neuritis, iridocyclitis, episcleritis, retinitis, uveitis
Cardiovascular	Myocarditis, pericarditis, heart failure, arrhythmias, hemodynamic instability
Dermatological	Photosensitive hyperpigmentation, intertriginous aphthous-like ulcers, vesiculobullous dermatosis
Renal	Nephritis, acute renal failure
Other	Bleeding dyscrasias, pneumonia, respiratory failure, hepatitis, pancreatitis, syndrome of inappropriate secretion of antidiuretic hormone (SIADH), hypoadrenalism

Adapted from Rajapakse et al. [20]

Some of the atypical manifestations are more in common in certain groups. For instance, meningoencephalitis and vesiculobullous dermatosis are observed more frequently in children and infants, respectively.[21, 22]

3C. High-risk Groups

CHIKV can affect women and men of all ages. Clinical presentation is thought to vary by age, however, with the very young (neonates) and the elderly being at greater risk for more severe disease.[23-26] In addition to age, comorbidities (underlying diseases) have also been identified as a risk factor for poor disease ontcome.[8, 23, 24, 27]

Most CHIKV infections that occur during pregnancy will not result in the virus being transmitted to the fetus.[25, 28] There have been rare reports of spontaneous abortions following CHIKV infection in the mother, however.[26] The highest transmission risk appears to be when women are infected during the intrapartum period[29] The vertical transmission rate is as high as 49% during this period. Infants are typically asymptomatic at birth and then develop fever, pain, rash, and peripheral edema. Those infected during the intrapartum period may also develop neurologic disease (e.g., meningoencephalitis, white matter lesions, brain swelling, and intracranial hemorrhage), hemorrhagic symptoms, and myocardial disease.[30] Laboratory abnormalities included raised liver function tests, reduced platelet and lymphocyte counts, and decreased prothrombin levels. Neonates who suffer from neurologic disease often develop long-term disabilities.[31] There is no evidence that the virus is transmitted through breast milk.[25]

Older adults are more likely to suffer from severe atypical disease and death. Individuals >65 years had a 50-fold higher mortality rate when compared to younger adults (<45 years old).[23] Although it is unclear why older adults are at increased risk for more severe disease, it may be due to the frequency of concomitant underlying diseases or decreased immunlogic response.[23]

CLINICAL

3D. Differential Diagnosis

Fever with or without arthralgia is a very common manifestation of several other diseases. CHIK may not have the typical manifestations or it may coexist with other infectious diseases such as dengue fever or malaria. Diseases that can be considered in the differential diagnoses may vary based on pertinent epidemiologic features such as place of residence, travel history, and exposure (Table 3).

Table 3. **Diseases or agents in the differential diagnosis of CHIK.**

Disease or agent	Presentation
Malaria	Periodicity of fever and alteration of consciousness
Dengue fever	Fever and two or more of the following, retro-orbital or ocular pain, headache, rash, myalgia, arthralgia, leucopenia, or hemorrhagic manifestations. See section and table below for more information on dengue
Leptospirosis	Severe myalgia localized to calf muscles with conjunctival congestion/or subconjunctival hemorrhage with or without jaundice or oliguria. Consider history of contact with contaminated water
Alphaviral infections (Mayaro, Ross River, Barmah Forest, O'nyong nyong, and Sindbis viruses)	Similar clinical presentation as CHIK; utilize travel history and known areas of Mayaro in the Americas

(Continued)

Table 3. **Diseases or agents in the differential diagnosis of CHIK.** (Cont.)

Disease or agent	Presentation
Post-infectious arthritis (including rheumatic fever)	Arthritis of one or more, typically larger joints due to an infectious disease such as Chlamydia, shigella, and gonorrhea. Rheumatic fever is seen more commonly in children as migratory polyarthritis predominantly affecting large joints. Consider antistreptolysin O (ASO) titer and history of sore throat with Jones criteria for rheumatic fever
Juvenile rheumatoid arthritis	Abrupt onset of fever and subsequent joint involvement in children

Overlap and Confusion with Dengue Fever:

CHIK has to be distinguished from dengue fever, which has the potential for much worse outcomes, including death. The two diseases can occur together in the same patient. Observations from previous outbreaks in Thailand and India have characterized the principal features distinguishing CHIK from dengue fever. In CHIK, shock or severe hemorrhage is very rarely observed; the onset is more acute and the duration of fever is much shorter. In CHIK, maculopapular rash also is more frequent than in dengue fever (Table 4). Although people may complain of diffuse body pain, the pain is much more pronounced and localized to the joints and tendons in CHIK, in comparison of dengue fever.

CLINICAL

Table 4. **Comparison of the clinical and laboratory features of chikungunya and dengue virus infections.**[a]

Clinical and laboratory features	Chikungunya virus infection	Dengue virus infection
Fever (>102°F or 39°C)	+++	++
Myalgias	+	++
Arthalgias	+++	+/-
Headache	++	++[b]
Rash	++	+
Bleeding dyscrasias	+/-	++
Shock	-	+
Leukopenia	++	+++
Neutropenia	+	+++
Lymphopenia	+++	++
Elevated hematocrit	-	++
Thrombocytopenia	+	+++

[a] Mean frequency of symptoms from studies where the two diseases were directly compared among patient seeking care; +++ = 70-100% of patients; ++ = 40-69%; + = 10-39%; +/- = <10%; - = 0% [32, 33]

[b] Often retroorbital

Table modified from Staples et al.[34]

3E. Subacute and Chronic Disease

After the first 10 days, most patients will feel an improvement in their general health and joint pain. Following this period, however, a relapse of symptoms can occur, with some patients complaining of various rheumatic symptoms, including distal polyarthritis, exacerbation of pain in previously injured joints and bones, and subacute hypertrophic tenosynovitis in wrists and ankles. This is most common two to three months after their illness onset. Some patients can also develop transient peripheral vascular disorders, such as Raynaud's syndrome. In addition to physical symptoms, the majority of patients will complain of depressive symptoms, general fatigue, and weakness.[13]

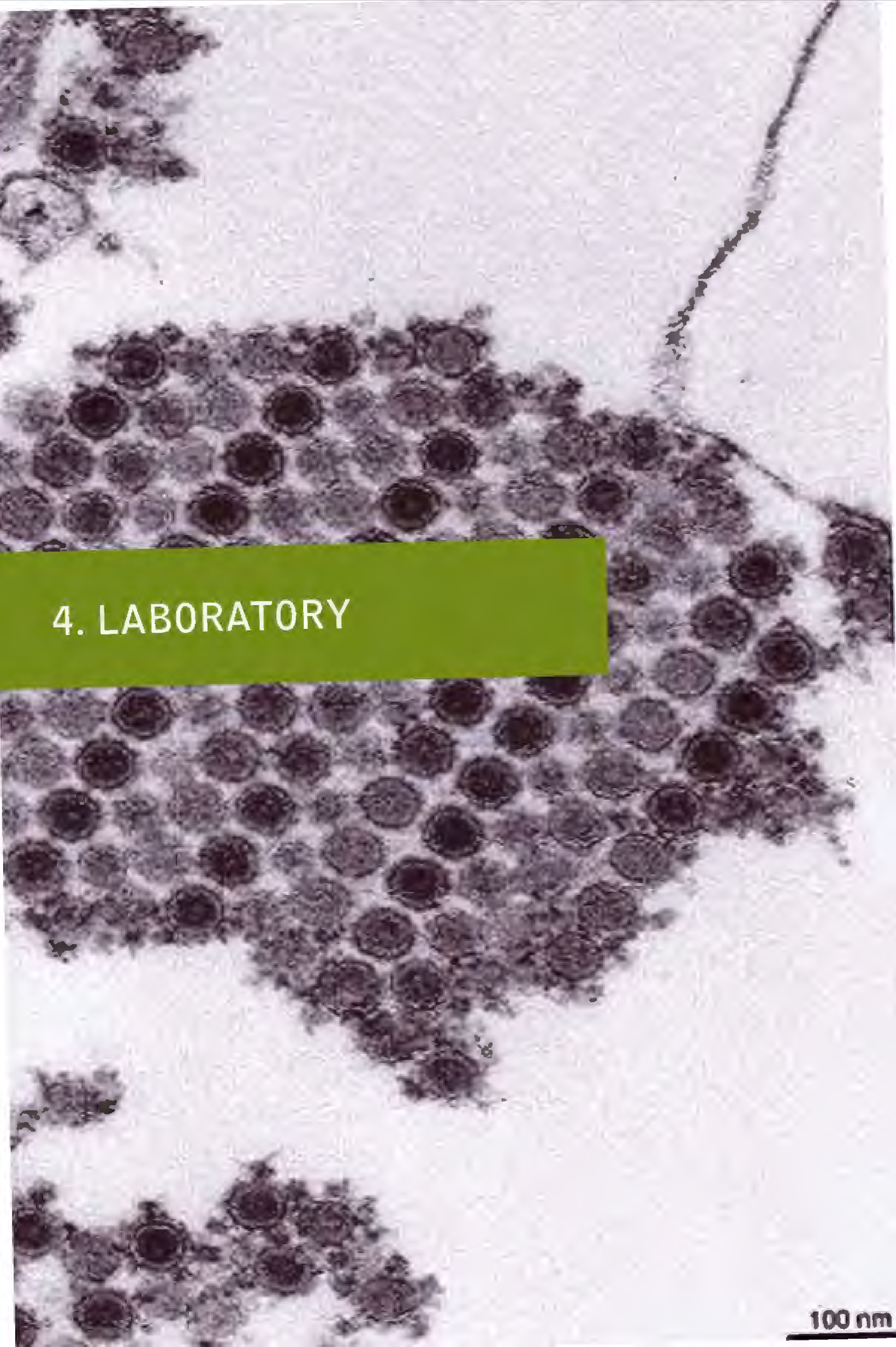
Chronic disease is defined by symptoms that persist for more than three months. The frequency of persons reporting persistent symptoms varies substantially by study and the time that had elapsed between symptom onset and follow-up. Studies from South Africa note that 12%–18% of patients will have persistent symptoms at 18 months and up to 2 to 3 years later.[35, 36] From more recent studies in India, the proportion of patients with persistent symptoms at 10 months was 49%.[37] Data from La Réunion have found that as many as 80%–93% of patients will complain of persistent symptoms 3 months after disease onset; this decreases to 57% at 15 months and to 47% at 2 years[38, 39] (F. Simone, Dept of Infectious Diseases and Tropical Medicine, Laveran Military Hospital, Marseilles, France, *personal communication*).

The most common persistent symptom is inflammatory arthralgias in the same joints that were affected during the acute stages. Usually, there is no significant change in laboratory tests and x-rays of the affected areas. However, some individuals will go onto develop destructive arthropathy/arthritis resembling rheumatoid or psoriatic arthritis.[40] Other symptoms or complaints of the chronic phase of the disease can include fatigue and depression.[6] Risk factors for non-recovery are older age (> 45 years), pre-existing joint disorders, and more severe acute disease.[38, 41]

CLINICAL

Summary of Clinical Section

- Acute stage is symptomatic in most people and causes acute fever, distal polyarthralgias, and occasional rash.
- Severe and lethal forms are more frequent among patients older than 65 years and/or with underlying chronic diseases.
- Maternal-fetal transmission is possible among pregnant women, with the highest risk for severe infection in the neonates during the antepartum period.
- Most patients initially will have severe and incapacitating joint symptoms; many will go on to develop long-lasting rheumatism, fatigue, and depression resulting in an impaired quality of life for months to years.



4. LABORATORY

4A. Types of Laboratory Tests Available and Specimens Required

Three main types of laboratory tests are used for diagnosing CHIK: virus isolation, reverse transcriptase-polymerase chain reaction (RT-PCR), and serology. Samples collected during the first week after onset of symptoms should be tested by both serological (immunoglobulin M [IgM] and G [IgG] ELISA) and virological (RT-PCR and isolation) methods. Specimens are usually blood or serum, but in neurological cases with meningoencephalitic features, cerebrospinal fluid (CSF) may also be obtained. Limited information is available for the detection of virus by isolation or RT-PCR from tissues or organs. In suspected fatal cases, virus detection can be attempted on available specimens.

Selection of the appropriate laboratory test is based upon the source of the specimen (human or field-collected mosquitoes) and the time of sample collection relative to symptom onset for humans.

Virus Isolation

Virus isolation can be performed on field collected mosquitoes or acute serum specimens (≤8 days). Serum obtained from whole blood collected during the first week of illness and transported cold (between 2°–8°C or dry ice) as soon as possible (within 48 hours) to the laboratory can be inoculated into a susceptible cell line or suckling mouse. CHIKV will produce typical cytopathic effects (CPE) within three days after inoculation in a variety of cell lines, including Vero, BHK-21, and HeLa cells. Virus isolation can be performed in T-25 flasks or shell vials (see Appendix A). Recent data suggest that isolation in shell vials is both more sensitive and produces CPE earlier than conventional isolation in flasks[42] CHIKV isolation must be confirmed either by immunofluorescence assay (IFA), using CHIKV-specific antiserum, or by RT-PCR of the culture supernatant or mouse brain suspension. Virus isolation must only be carried out in biosafety level 3 (BSL-3) laboratories to reduce the risk of viral transmission.

RT-PCR

Several RT-PCR assays for the detection of CHIKV RNA have been published. Real time, closed system assays should be utilized, due to their increased sensitivity and lower risk of contamination. The Arboviral Diagnostic Laboratory within DVBD, CDC routinely utilizes the published assay in Appendix B,[43] which demonstrates a sensitivity of less than 1 pfu or 50 genome copies. Serum from whole blood is used for PCR testing as well as virus isolation.

Serological Tests

For serological diagnosis, serum obtained from whole blood is utilized in enzyme-linked immunosorbent assay (ELISA) and plaque reduction neutralization testing (PRNT). The serum (or blood) specimen should be transported at 2°–8°C and should not be frozen. Serologic diagnosis can be made by demonstration of IgM antibodies specific for CHIKV or by a four-fold rise in PRNT titer in acute and convalescent specimens. IgM antibodies specific for CHIKV are demonstrated by using the IgM antibody capture ELISA (MAC-ELISA),[44] followed by the PRNT (detailed protocols for IgM and IgG ELISAs shown in Appendix C). As of 2010, there were no World Health Organization (WHO)

validated commercial IgM ELISAs available. PRNT is required to confirm the MAC-ELISA results, since cross-reactivity in the MAC-ELISA between some members of the Semliki Forest virus (SFV) serogroup has been observed. PRNT testing, whether used to confirm the MAC-ELISA or to demonstrate a four-fold rise in acute/convalescent specimens, should always include other viruses within the SFV serogroup (e.g., Mayaro virus) to validate specificity of reactivity. In situations where the PRNT assay is not available, other serological tests (e.g. hemaglutination inhibition [HI]) can be used to identify a recent alphavirus infection; however, PRNT is required to confirm a recent CHIKV infection.

An acute phase serum should be collected immediately after the onset of illness and the convalescent phase serum 10–14 days later. CHIKV-specific IgM and neutralizing antibodies normally develop towards the end of the first week of illness. Therefore, to definitively rule out the diagnosis, convalescent samples should be obtained on patients whose acute samples test negative.

Collection, Storage, and Transportation of Samples

Proper collection, processing, storage, and transportation of the specimens are essential aspects of the laboratory diagnosis.

Collection of samples for serology, isolation and molecular diagnosis:

Sample: Serum
Time of collection: Acute, within the first eight days of illness; convalescent, 10–14 days after acute specimen collection.

To collect serum:

- Aseptically collect 4–5 ml of venous blood in a tube or a vial.
- Allow blood to clot at room temperature, centrifuge at 2,000 rpm to separate serum. Collect the serum in a clean dry vial.
- All clinical samples should be accompanied by their clinical and epidemiological information.

Other types of specimens for laboratory investigation:

Specimens:

- CSF in meningo-encephalitis cases.
- Synovial fluid in arthritis with effusion.
- Autopsy material – serum or available tissues.

[Note: Mosquitoes collected in the field will also be handled using the same techniques described here]

Transportation of Samples:

- Transport specimens to the laboratory at 2°–8°C (icebox) as soon as possible.
- Do not freeze whole blood, as hemolysis may interfere with serology test results.
- If a delay greater than 24 hours is expected before specimens can be submitted to the laboratory, the serum should be separated and stored at refrigerated temperature.
- Serum samples for virus isolation and molecular diagnosis should be stored frozen (at –20°C for short-term storage or at –70°C for long-term storage).

LABORATORY

4B. Laboratory Surveillance

Prior to identification of CHIKV in a country, laboratory surveillance should be conducted on three sets of samples, as follows: 1) dengue-negative specimens where the patient exhibits severe joint pain; 2) samples with clinically compatible illness from new geographic areas without active dengue circulation; 3) clusters of febrile illness with severe joint pain. The following table (Table 5) outlines the ideal tests to be performed in various epidemiological settings.

Table 5. **Laboratory surveillance for Chikungunya virus by epidemiologic scenario.**

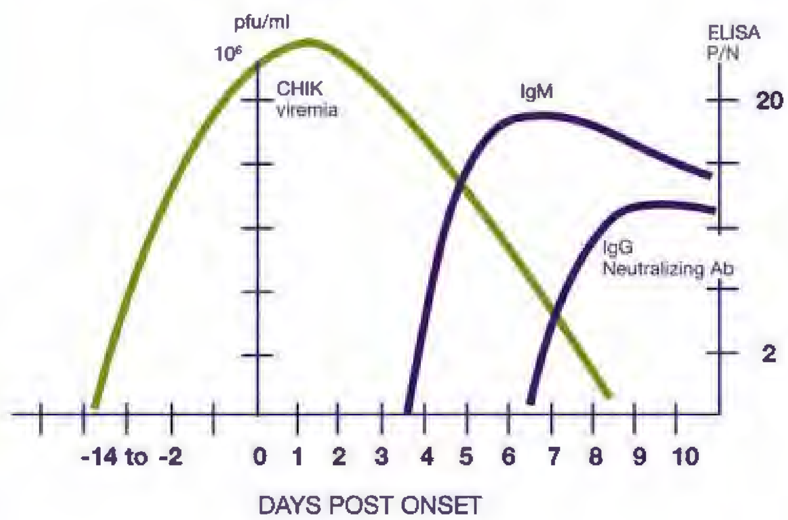
Epidemiological scenario	Testing to be performed	Samples to test
No signs of transmission	IgM ELISA, IgG ELISA	All samples from patients exhibiting clinically compatible illness
Suspect CHIKV illness	IgM ELISA, IgG ELISA, real-time RT-PCR, virus isolation, PRNT	All samples from patients exhibiting clinically compatible illness
Continued transmission	IgM ELISA, IgG ELISA, real-time RT-PCR; limited virus isolation	Subset samples from classical CHIK cases, as determined by lab constraints and epidemiological status; Samples from all atypical or severe cases should be tested
Periodic outbreaks (once CHIKV has been detected in an area) or active surveillance in areas near CHIKV transmission	IgM ELISA, IgG ELISA, real-time RT-PCR; limited virus isolation	Subset of samples from classical CHIK cases, as determined by lab constraints and epidemiological status; samples from all atypical or severe cases should be tested

During the initial introduction of CHIKV into a new region, comprehensive testing should be completed to confirm that CHIKV is the etiological agent. After CHIKV has been identified, limited testing (not testing all specimens or performing fewer assay types) can be considered depending upon the capacity of the lab and the epidemiological situation.

4C. Interpretation and Reporting of Results

Figure 2 shows typical viremia and antibody response in humans and Table 6 describes the typical results of testing samples at various time points.

Figure 2. Viremia and immune response following Chikungunya virus infection.



LABORATORY

Table 6. **Typical results of samples tested at various time points post-infection.**

Days post illness onset	Virus testing	Antibody testing
Day 1-3	RT-PCR = Positive Isolation = Positive	IgM = Negative PRNT = Negative
Day 4-8	RT-PCR = Positive Isolation = Negative	IgM = Positive PRNT = Negative
>Day 8	RT-PCR = Negative Isolation = Negative	IgM = Positive PRNT = Positive

The following laboratory test results would confirm a recent CHIKV infection:

- Isolation of CHIKV, including confirmatory identification (either IFA, RT-PCR, or sequencing).
- Detection of CHIKV RNA by real time RT-PCR.
- Identification of a positive IgM result in a patient with acute symptoms of CHIK, followed by the demonstration of CHIKV-specific antibody determined by PRNT with viruses in the SFV serogroup.
- Demonstration of seroconversion or a four-fold rise in PRNT, HI, or ELISA titers (again using other SFV serogroup viruses) between acute and convalescent specimens.

Autochthonous cases should be reported to WHO, in collaboration with an epidemiologist, according to International Health Regulations (IHR) (see section 6F).

LABORATORY

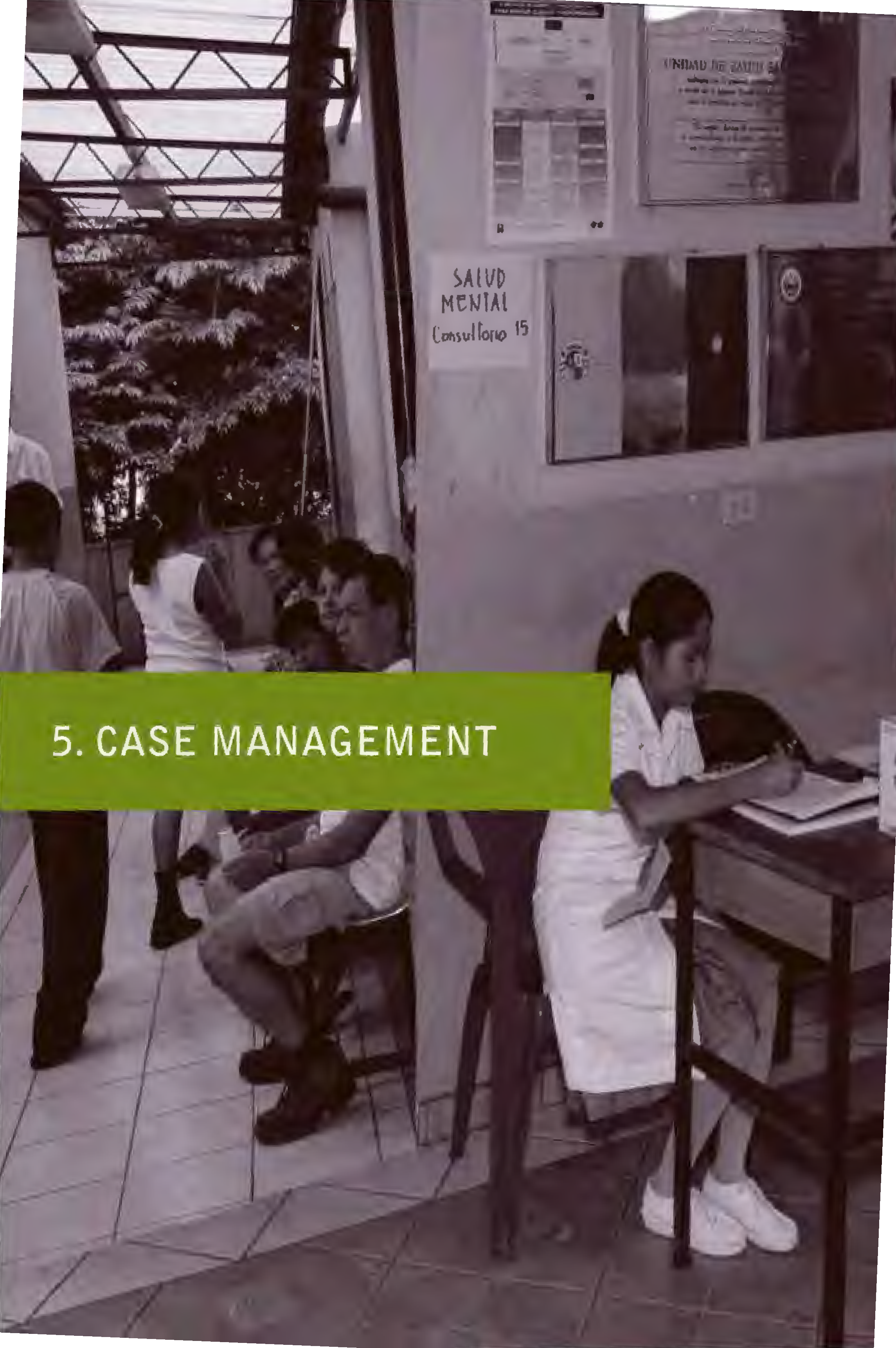
4D. Laboratory Network for Diagnosing CHIKV

Currently DVBD, CDC can provide diagnostic testing for CHIKV infection. Reagents and consultations can also be provided by CDC and the Public Health Agency of Canada. Depending on the availability of resources and the epidemiologic situation, PAHO and CDC will be working together in the near future to improve CHIKV detection in the Region by providing training and reagents to existing dengue (RELDA) and other arbovirus laboratories in the Americas. Furthermore, proficiency testing is planned to ensure testing quality in the Region. A contingency plan will be developed to ensure that all laboratories capable of performing testing in the Americas have an adequate supply of reagents and protocols.

Summary of Laboratory Section

- Both molecular and serologic techniques are available for the laboratory diagnostic evaluation of CHIKV infection.
- During an outbreak, laboratories will need to develop, with other public health partners, sample triage plans to avoid laboratory overload.
- Laboratories have a key role in the surveillance for CHIKV introduction and spread; ongoing training of laboratories for CHIK detection is needed throughout the Region.
- Collaboration is important, in order for network partner (public health) labs to be able to share materials.
- Reference laboratories in the Region will play a significant role in reagent production and in providing laboratory confirmation of suspected CHIK cases.

5. CASE MANAGEMENT



5A. Treatment

There is no specific antiviral drug treatment for CHIK. Symptomatic treatment is recommended after excluding more serious conditions like malaria, dengue, and bacterial infections.

Acute Disease

Treatment is symptomatic or supportive, comprised of rest and the use of acetaminophen or paracetamol to relieve fever, and ibuprofen, naproxen, or another non-steroidal anti-inflammatory agent (NSAID) to relieve the arthritic component of the disease. Using aspirin is not advised because of the risk of bleeding in small number of patients and the risk of developing Reye's syndrome in children younger than 12 years of age. In patients with severe joint pains that are not relieved by NSAID, narcotics (e.g., morphine) or short-term corticosteroids can be used after evaluating the risk-benefit of these treatments. Patients should be advised to drink plenty of fluids to replenish fluid lost from sweating, vomiting, and other insensible losses.

Subacute and Chronic Disease

While recovery from CHIK is the expected outcome, convalescence can be prolonged (sometimes up to a year or even more) and persistent joint pain may require pain management, including long-term anti-inflammatory therapy. Although an older study suggested that chloroquine phosphate offered some benefit,[45] a recent donble-blind, placebo-controlled randomized trial found it to be of no real value treating joint symptoms.[46] Disabling peripheral arthritis that has a tendency to persist for months, if refractory to other agents, may occasionally respond to short-term corticosteroids.[38] To limit the use of oral corticosteroids, local injections (intra-articular) of corticosteroids or topical NSAID therapy can be used. In patients with refractory joint symptoms, alternative therapies such as methotrexate can be evaluated. In addition to pharmacotherapy, cases that have prolonged arthralgia and joint stiffness may benefit from a program of graduated physiotherapy. Movement and mild exercise tend to improve morning stiffness and pain, but heavy exercise may exacerbate symptoms.

5B. Patient Isolation Recommendations

To prevent the infection of others in the household, the community, or the hospital, a patient with acute CHIK should avoid being bitten by ***Ae. aegypti*** or ***Ae. albopictus*** mosquitoes during the viremic phase, which is usually the first week of illness. As these mosquitoes bite during daytime, from dawn to dusk or even after dark in the presence of artificial light, staying under an insecticide-treated (IT) bednet or remaining in place with intact screens is highly recommended. Furthermore, physicians or health care workers who visit CHIK-infected patients at home should take care to avoid being bitten by mosquitoes by using insect repellent and wearing long sleeves and pants.

One hospital-associated infection of CHIK has been identified in a health-care provider who had an accidental needle stick from a patient with CHIK.[47] Several laboratory workers also have contracted CHIKV infection after handling infected blood.[48] These exposures indicate that direct contact transmission can occur. However, other modes of transmission, such as through respiratory droplets or particles, have not been documented.

5C. Health Care and Hospital Surge Capacity

At the peak of one recent outbreak, 47,000 suspected cases were identified in a single week among a population of 766,000.[27] There also can be an accumulation of patients with symptoms who seek more long-term care. With that potential volume of cases per week, huge demands are likely to be placed on the health care system during outbreaks of the disease. A number of steps similar to those for pandemic influenza preparedness should be considered by health care facilities preparing for and during a CHIK outbreak. Triage systems should be considered at various levels of health care to facilitate the flow of patients during an outbreak.

Prior to the introduction of CHIKV, the following should be considered (adapted from PAHO and U. S. Department of Health and Human Services (HHS) Influenza Pandemic Plan[49, 50]):

- Develop and implement methods for identifying and investigating potential introduction of CHIKV within existing surveillance systems (e.g., surveillance system for dengue).
- Inform health care providers and public health officials about the potential threat of CHIKV, and educate them about the clinical presentation, diagnosis, and management of cases at health care facilities.

CASE MANAGEMENT

- Develop planning and decision-making structures for responding to a potential outbreak at health care facilities.

- Develop institutional plans to address disease surveillance, hospital communications, education and training, triage and clinical evaluation, facility access, occupational health, surge capacity (beds and access to care), supply chain, and access to critical inventory needs.

Following the introduction of CHIKV into an area, health care facilities should:

- Activate institutional plans with assistance from the Ministry of Health.

- Ensure rapid and frequent communication within health care facilities and between health care facilities and health departments.

- Implement surge-capacity plans that address staffing, bed capacity, consumable and durable supplies, and continuation of essential medical services (see section on Health care Planning in the PAHO and HHS Pandemic Influenza Plan for further considerations[49, 50]).

Effective triage systems at various levels of health care may help to decrease the potential burden of a CHIK outbreak on the health care system. Regardless of the level of medical care available at the triage location, a key measure that needs to be considered at all levels of health care is the institution of appropriate mosquito control measures in the immediate area. If this is not done, patients acutely ill with CHIK can serve as a source of subsequent infections for other patients and for health care workers via mosquito transmission. Furthermore, consideration should be given to establishing areas where patients with suspected CHIK infection are seen and, if necessary, hospitalized (e.g., establish CHIK wards with screens and/or bednets). Finally, consideration should be given to the safety of health care workers. During a previous outbreak, up to one-third of health care workers became infected, further taxing already overburdened and stretched resources.[9]

'Guiding principles for managing acute stage of the disease' has been previously described in detail in WHO's "Guidelines on Clinical Management of Chikungunya Fever". [51] Key information, including triage considerations, from that document is summarized here.

Who should seek medical care?

- Anyone with neurologic signs or symptoms including irritability, drowsiness, severe headaches, or photophobia.
- Anyone with chest pain, shortness of breath, or persistent vomiting.
- Anyone with a fever persisting for more than five days (indicative of another illness like dengue).
- Anyone who develops any of the following, especially once the fever subsides:
  - intractable severe pain,
  - dizziness, extreme weakness, or irritability,
  - cold extremities, cyanosis,
  - decreased urine output, and
  - any bleeding under the skin or through any orifice.
- Women in the last trimester of pregnancy, newborns, and persons with chronic underlying disease, as they or their offsprings are at risk for more severe disease.

Triage at point of first contact (Primary or ambulatory/urgent care)

- Rule out other illnesses by history, clinical examination, and basic laboratory investigations, including but not limited to complete blood count (CBC), liver function tests, and electrolytes. Be careful to evaluate if patient has warning signs for severe dengue or malaria. If these signs are present, refer patient immediately to a hospital.
- Assess for dehydration and provide proper rehydration therapy as needed.
- Evaluate hemodynamic status and stabilize and immediately refer patients with delayed capillary refill, narrow pulse pressure, hypotension, oliguria, altered sensorium, or bleeding manifestations.
- Treat symptomatically (paracetamol/acetaminophen).
- For those with prolonged joint pain (after three days of symptomatic treatment) consider more aggressive pain management, such as morphine and short-term corticosteroids.

- Consider referral for patients with increased risk of a poor outcome (persons older than 60 years, those with chronic disease, pregnant women, and newborns).

Triage at the secondary level (district or local hospital)

- Treat symptomatically (according to previous treatments).
- Investigate person for renal failure, neurologic signs and symptoms, hepatic insufficiency, cardiac illness, thrombocytopenia, and malaria.
- Evaluate hemodynamic status and assess for dehydration; provide proper supportive care and rehydration therapy as needed.
- Consider cerebral spinal tap if meningitis is suspected.
- Collect blood for serologic testing of CHIK and other diseases in the differential diagnosis (e.g., dengue).
- Review history of present illness and evaluate if patient has warning signs for severe dengue. If present, administer supportive care in a unit that can monitor vital signs on an hourly basis during the critical phase.
- Refer patients with any of the following conditions to a higher level health center: pregnancy, oliguria/anuria, refractory hypotension, significant clinical bleeding, altered sensorium, meningoencephalitis, persistent fever of more than one week's duration, and signs of decompensation of underlying diseases.

Triage at the tertiary care level (advanced care centers or centers with infectious disease specialists)

- Ensure that all the above-mentioned procedures have been completed and that a comprehensive medical team is available to assist in managing patients with severe or atypical disease.
- Collect blood sample for serology and/or RT-PCR (see Laboratory section for more specific on CHIK testing).

- Consider the possibility of other rheumatic (e.g., rheumatoid arthritis, gout, rheumatic fever) or infectious diseases (e.g., viral or bacterial meningoencephalitis).
- Treat serious complications (e.g., bleeding disorder with blood components, acute renal failure with dialysis).
- Assess disability and recommend rehabilitative procedures.
- Given the severity of the pain and the potential long-term pain with CHIK, pain management and psychological assistance should be made available and consideration given to develop chronic pain management protocols, teams, and centers. Autopsies should be considered on all deceased patients, with involvement of pathologists.

5D. Blood, Organ, and Tissue Safety

Blood-borne transmission is possible. There are documented cases that include infection of laboratory personnel handling infected blood and of a health care worker drawing blood from an infected patient.[47, 48] These cases support the belief that CHIKV is able to be transmitted through blood products.

To determine the impact of CHIKV on blood supply safety consider: 1) incidence of viremia among blood donors (which may vary depending on the time of the outbreak); 2) clinical impact on transfusion recipients who become infected; 3) availability of measures to reduce transfusion transmission (e.g., nucleic acid amplification testing (NAT) or photochemical pathogen inactivation treatment); 4) availability of an alternative blood supply (from non-affected areas); and 5) the cost incurred by adopting these measures.[52]

In addition to asking the local health-care community to promote optimal use of blood components, possible considerations for blood safety in areas with CHIKV introduction could include:[53]

- Continue to obtain blood donations from local persons until an unacceptable incidence or prevalence[a] of infection is reached in the community.
- Screen blood donors for symptoms prior to donation.
- Asking donors to report any illness they experience after donating blood, while holding on to the blood donations for several days (e.g, 2-5 days) prior to releasing it.
- If feasible, cease all blood donations in an area of known CHIKV infections and import blood products from uninfected areas.
- Institute screening (e.g., NAT) of the blood supply for CHIKV. This will require a preexisting platform and regulatory clearance, and is unlikely to be available in most areas.

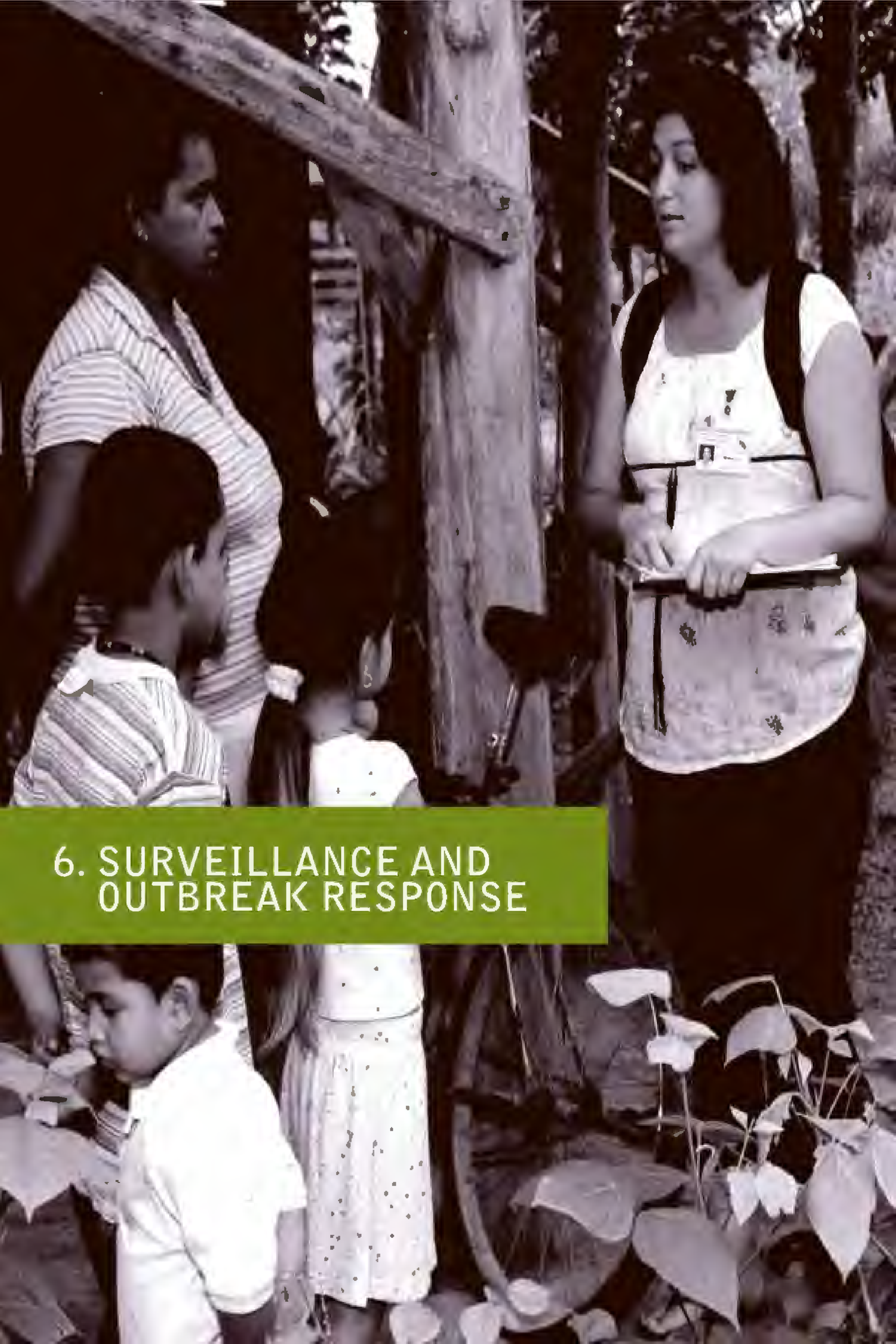
Similar measures should be considered for organ and tissue (grafts) transplantations.

CASE MANAGEMENT

[a] To be determined by blood banks and public health officials in the area.

Summary of Case Management Section

- Treatment for CHIK is supportive, using anti-pyretics, optimal analgesics, and fluids.
- Acutely infected patients need to be protected against mosquito bites to prevent further disease spread at home, in the community, and in the health care facility.
- Because CHIK will place a large burden on the community, including on all levels of the health care system, well-established protocols and plans need to be developed in advance to assist in the triage, care, and rehabilitation of patients.



6. SURVEILLANCE AND OUTBREAK RESPONSE

The main objective of surveillance is to detect, in a timely manner, cases of CHIK in the Americas. Early detection will allow for proper response and characterization of the outbreak and identification of the viral strains circulating.

6A. Modes of Surveillance

Multiple modes of surveillance can be considered to determine if CHIK may have been introduced to an area, to track the disease once introduced, or to follow the disease once it has been established.

1. Preparedness phase

Reinforce existing febrile syndromic surveillance sentinel sites so they can detect CHIK cases. A percentage of patients presenting with fever and arthralgia or fever and arthritis with no known etiology (e.g., negative test for malaria or dengue), should be tested for CHIK at the national reference laboratory (See Section 4 for more details on proposed laboratory surveillance testing). To ensure adequate laboratory testing and surveillance capacity, laboratories should be aware of the laboratory network set up for testing and eventual distribution of supplies.

2. Response phase

Introduction

Once an autochthonous case of CHIK is detected, an in-depth epidemiologic investigation must be conducted to:

- Track viral spread.
- Monitor for possible introduction into surrounding areas.
- Describe key epidemiologic and clinical features.
- Assess clinical severity and impact on society (e.g., days missed from work, school closures, etc.).
- Identify risk factors for infection or severe disease.
- Identify circulating CHIKV lineages.

These efforts will be the basis for developing effective control measures.

Active, passive, and laboratory surveillance should be used to calculate and monitor indicators such as: incidence, rate of spread, rate of hospitalization (per infections), proportion of severe disease, mortality ratios, and disability rates.

Sustained transmission

Once the virus has been identified throughout a country, scaling back of the level of testing and active surveillance can be considered (e.g., testing only a fraction of suspect cases or testing severe or atypical cases, newborns, cases identified in new regions) to avoid unnecessary costs in resource-limited settings. However, ongoing surveillance should be continued to monitor changes in the epidemiology and ecology of CHIKV transmission. Any changes in surveillance at the national level should be readily communicated to other surveillance and prevention partners, such as vector control specialists, to ensure the quality and uniformity of the data collected.

6B. Case Detection

Clinicians should consider CHIK in the differential diagnosis for individuals who are presenting with fever and arthralgias that are not explained by another etiology or have an atypical presentation, e.g., an atypical dengue presentation with more severe joint pain or conjunctivitis. The index of suspicion should be heightened for a traveler or someone having contact with a traveler who has recently returned from an area with ongoing CHIKV infections (to obtain updated information on location of CHIK outbreaks visit http://www.who.int/csr/don/en/index.html or http://wwwnc.cdc.gov/travel/default.aspx).

Laboratory personnel should consider CHIK if there is a low proportion of samples that are seropositive for an etiology that has a similar clinical presentation, like dengue, or if there are a number of synovial fluid samples that are sterile on bacterial culture.

Public health authorities should be alerted to small clusters of disease (fever and arthralgia or arthritis) associated with a traveler returning from a CHIK endemic area or an increase in the number of hospital visits for fever and arthralgia or arthritis occurring in a localized area in a short time.

6C. Case Definition

Suspect case: a patient with acute onset of fever >38.5°C (101.3°F) and severe arthralgia or arthritis not explained by other medical conditions, and who resides or has visited epidemic or endemic areas within two weeks prior to the onset of symptoms.

Confirmed case: a suspect case with any of the following CHIK specific tests:

- Viral isolation.
- Detection of viral RNA by RT-PCR.
- Detection of IgM in a single serum sample (collected during acute or convalescent phase).
- Four-fold increase in CHIKV-specific antibody titers (samples collected at least two to three weeks apart).

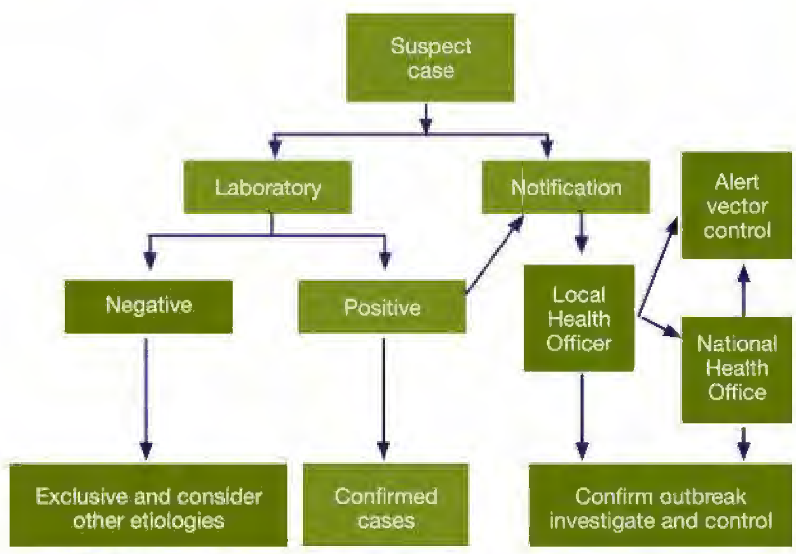
During an epidemic, all patients need not be subjected to confirmatory tests as above. An epidemiologic link can be sufficient.

An evaluation of the sensitivity and specificity for clinical criteria for CHIKV infection was done during a large outbreak of the disease.[54] The combination of fever and polyarthralgias had the best sensitivity and specificity at 84% and 89%, respectively, and allowed for the correct classification 87% of individuals with serologically confirmed CHIKV infection.

6D. Case Reporting

CHIK is not a notifiable disease in most countries. However, depending on the epidemiologic situation, each country must determine independently when CHIK should be a disease of mandatory reporting. Occurrence of suspect cases could indicate a possible outbreak and, therefore, should be immediately reported to the nearest health authority in accordance with the IHR guidelines. Prior to the introduction of CHIK into an area, clinicians should report any suspect or confirmed travel-related cases to local public health officials who, in turn, should report them to a regional level and then on to a national level, where the information should be summarized and shared with stakeholders (Figure 3). In addition, other key partners, such as vector control management teams, should be notified.

Figure 3. **Scheme for notification of a suspected outbreak of CHIK.**



6E. Epidemiologic Reports

Ideally, epidemiologic reporting should be established at the national level, with the support of local and regional public health officials. The types and number of epidemiologic reports will likely evolve during the course of the outbreak to reflect the types of surveillance that are performed in an area.

Following the introduction of CHIK into an area, a line list of suspect and laboratory confirmed cases should be kept and updated daily. Reporting should be coordinated at the national level by establishing a web-based line list, if possible, that contains a few required variables and additional variables as needed. A standardized case report form, including demographic, epidemiologic and laboratory information, should be developed quickly and shared with key partners to help facilitate the collection of information (See Appendix D for an example). At the national level, there should be clearly defined cutoffs in

terms of presenting and closing the data on a daily basis. In addition to case count by location and timing, reporting on disease severity (hospitalization, mortality), number of hospital beds occupied per day, and trends in cases based on syndromic surveillance can be considered as ways to present the data. The national level data should be communicated back to the collecting districts, as well as to the press and other public health and partner agencies that participate in the control efforts (see Section 8, "Risk and Outbreak Communication" for more detail). Once a country has identified autochthonous transmission within its borders, it should activate its emergency operations center ("sala de situación") to serve as a source for rapid communication and decision making.

6F. International Health Regulations and Border Measures

International Health Regulations

A single imported case (i.e., a traveler) of CHIKV into the Americas would not necessarily constitute a public health emergency of international concern (PHEIC) under IHR,[55] although this case should be thoroughly investigated to minimize the risk of CHIK establishment in the country. However, suspicion of autochthonous CHIKV transmission in the Americas will meet PHEIC criteria and should be reported per IHR (see Appendix E for an example). Such an event would have a serious public health impact because of its potential to cause an epidemic with high attack rates among an immunologically naïve population, and because vectors are sufficiently abundant to potentially support permanent establishment of the virus and year-round transmission. The event would also be unusual for the Americas, since it would signal the appearance of a previously absent pathogen and a significant risk of international spread given the amount of travel between countries in the Region. Although CHIKV does not have a high mortality rate, it has high morbidity rates associated with persistent arthralgias that can lead to disability and a reduction in productivity. The establishment of CHIKV in a Member Country could also affect key national income sources, such as tourism. For example, La Réunion Island observed a 60% decline in tourism after its CHIKV outbreak.[56]

Any Member Country should thoroughly investigate any suspect CHIK case detected without a travel epidemiologic link to another country and rule out indigenous CHIKV transmission. PAHO recommends that Member Countries consider making the reporting of CHIK mandatory to enable and promote a timely response.

Border Measures

Closing borders due to suspected CHIKV cases would be counterproductive and it is not recommended by WHO. It also is inconsistent with the IHR, which emphasize detection and containment at the new source of transmission, rather than control at borders of entry. The costs associated with screening for CHIK at ports of entry outweigh the benefits. It is insufficiently sensitive and specific and too expensive to be a tool for preventing CHIKV introduction and spread. The anticipated prevalence among travelers coming from areas of the world with CHIKV activity is low, symptoms are non-specific, and screening would yield a low positive predictive value. The reported experience of entry screening for CHIKV in Taiwan validates this point. During 2006, more than 11.7 million passengers arrived in Taiwan. Of these passengers, 6,084 were identified as having fever using thermal infrared imaging cameras; laboratory testing of passengers detected 44 cases of dengue fever, 13 cases of shigellosis, 1 case of malaria, 1 case of paratyphoid fever, and 1 case of CHIK (JW Hsieh, Centers for Disease Control, Ministry of Health, Taiwan,*personal communication*, 2007).

Even disregarding the issue of cost and complexity of implementation, port of entry screening activities are unlikely to prevent or delay the importation of CHIKV. There is no evidence to support that requiring pilots or ship captains to complete health declarations, asking passengers to complete screening questionnaires, taking temperature measurements and engaging in other entry screening modalities effectively prevent CHIKV introduction and spread into the Americas. Member Countries should use their scarce public health resources on activities more likely to achieve intended results, including implementing sustainable vector control efforts, enhancing clinical surveillance for CHIKV disease, educating the public, and considering assisting affected Member Countries. For similar reasons, exit

screening is not recommended if Member Countries in the Americas confront CHIKV outbreaks within their borders.

Some jurisdictions outside the Americas have instituted mosquito abatement activities at international airports and spraying adulticides in the passenger cabins of arriving international flights as part of efforts aimed at preventing dengue importation. However, virus-infected mosquitoes arriving in passenger aircraft are not considered as significant sources of most arboviral importations. For arboviruses with a human-mosquito-human transmission cycle, the most important source of viral importation is the viremic traveler. In a region like the Americas, where competent vectors are already present in the majority of countries, mosquito abatement and vector surveillance efforts predominantly focused on international airports and seaports can be implemented by national authorities to prevent CHIKV importation, but PAHO does not recommend them. The exception would be if cases were being detected close to an international airport or seaport, or if suspected cases worked in or around these ports of entry. Routine vector control efforts consistent with IHR Article 22, which calls for eliminating vectors at facilities used by travelers at points of entry, should be implemented, but are not intended as a principal means of preventing CHIKV importation.

Similarly, in the presence of CHIK cases and local virus transmission, there is no need to place any restrictions on baggage, cargo, containers, goods, or postal parcels beyond usual practices; this will avoid unnecessary interference with international traffic in the absence of any identified public health benefit. It is advisable, however, to establish communications between public health authorities and conveyance operators (sea and air, cargo and passenger) and other port-based organizations, in case there is a need to implement a CHIKV communication campaign.

Countries may elect to distribute Travel Health Alert Notices (THANs) to international travelers if there is concern that CHIKV transmission is likely or if ongoing transmission has been detected. This information would offer guidance to travelers on how to reduce their risk of contracting CHIKV, steps to take for

reducing the likelihood that they will be bitten by mosquitoes, or seeking early diagnosis if they develop signs and symptoms compatible with CHIK fever. These messages could be relayed through online reservation systems, travelers' health clinics, travelers' health Web sites, and postings at international ports when outbreaks are occurring.

It will be important to monitor air travel patterns between countries where CHIKV is circulating and every other country or area in the Americas, in order to identify locations most at risk to virus introduction. In a preliminary analysis that was limited exclusively to direct flight data, scheduled commercial flight data shows that countries importing CHIKV had 23 times more total scheduled passenger seats originating from countries with CHIKV activity than did non-importing countries (CDC, unpublished). Subsequent analyses using passenger-specific data, which includes travel connections and actual passenger volume, could provide more accurate information on which to base a risk assessment of CHIKV importation.

Summary of Surveillance and Outbreak Response Section

- Epidemiological surveillance is key to the timely detection of cases and appropriate and rapid response with active participation from all stakeholders.
- CHIK surveillance should be built upon existing dengue surveillance (highlighting differences in clinical presentation).
- If autochthonous transmission of CHIK is identified, it must be reported immediately as a PHEIC under IHR.

7. VECTOR SURVEILLANCE AND CONTROL

In the absence of an effective CHIKV vaccine, the only tool available to prevent infection is reduction of human-vector contact. The primary vectors of CHIKV are *Ae. aegypti* and *Ae. albopictus*. *Ae. aegypti* is the principal vector in areas of Africa where the virus is considered to be endemic. However, *Ae. albopictus* was incriminated during recent epidemics, following introduction of the virus into temperate Europe[17] and some tropical areas of the Indian Ocean[27, 57] These outbreaks were associated with an adaptation of CHIKV strains to *Ae. albopictus*.[58, 59] Both *Ae. aegypti* and *Ae. albopictus* are present in the Americas (Figures 4 and 5). *Ae. aegypti* will likely be the most important vector in urban areas, and *Ae. albopictus* will likely play a more significant role in temperate areas and areas where it is already well established. Both mosquitoes could support the introduction of CHIKV strains into a variety of geographic areas in the Region. Therefore, vector control planning efforts should focus on suppression of both *Ae. aegypti* and *Ae. albopictus* populations to prevent the likelihood of CHIKV establishment and to lay the foundation for emergency intervennons in the event of an outbreak.

VECTOR

Figure 4. **Distribution of *Ae. aegypti* in the Americas.**[a]

[a] Adapted from Arias, 2002.[60]

Figure 5. **Approximate distribution of *Ae. albopictus* in the Americas.**[a]

[a] Adapted from Benedict et al. 2007.[61]

VECTOR

There are some significant differences between *Ae. aegypti* and *Ae. albopictus* that must be considered in developing surveillance and control procedures. *Ae. aegypti* is more closely associated with humans and their homes, and feeds preferentially on humans. Adult *Ae. aegypti* rest indoors, and its larval habitats are most frequently containers on the household premises. *Ae. albopictus* feeds readily on humans, but also utilizes a broader range of bloodmeal hosts;[62] its larvae occur in peridomestic habitats as well as surrounding natural habitats. *Ae. albopictus* can overwinter in the egg stage and, therefore, can occupy more temperate climates[63] than *Ae. aegypti*. These species have distinct morphological features, and the identification of specimens collected during surveillance and control programs in the Americas can be readily accomplished.[64, 65]

An effective, operational dengue control program provides the basis for CHIKV preparedness, because the biology and control procedures for *Ae. aegypti* are similar to those for *Ae. albopictus*. Surveillance and control recommendations developed for dengue management[66] as a component of the Integrated Management Strategy for Dengue Prevention and Control (IMS-Dengue) may be utilized and intensified in order to respond to a CHIKV introduction. Successful control programs require well trained professional and technical staff and sufficient funding. In addition, an independent quality assurance program should be incorporated into the integrated vector management (IVM) scheme.

To be successful, the CHIKV IVM program must include intersectoral participation (collaboration) at all levels of government and among health, education, environment, social development, and tourism agencies. IVM programs also benefit from participation of non-governmental organizations (NGOs) and private organizations. CHIKV control program must communicate with and mobilize the entire community.[67] In fact, the community's participation is an essential component of IVM.[68] To be effective, an IVM strategy must be developed and in place before CHIKV is introduced.

7A. Reducing the Risk of CHIKV

Components of an IVM program to reduce CHIKV risk include:

1. Vector Surveillance and Identification of High Risk Areas

In areas where dengue is endemic, a retrospective analysis of Dengue virus transmission during previous years should be conducted during the CHIKV planning phase to indicate the areas where CHIKV is expected to circulate (given the similarity in transmission cycles of these viruses). Areas can be stratified in terms of risk of transmission.[69] Stratification is then used to assign resources and priorities. For example, controlling or preventing CHIKV transmission in neighborhoods that traditionally have produced many cases of dengue should inhibit virus amplification and virus spread to nearby neighborhoods.

The program must have the ability to systematically collect surveillance data on relative densities of *Ae. aegypti* and *Ae. albopictus*. Surveillance methods for *Ae. aegypti* and *Ae. albopictus* are varied and include methods to monitor egg production, larval sites, pupal abundance, and adult abundance. These methods are reviewed in Chapter 5 of the WHO Dengue Guidelines.[66] New traps and sampling methods are being developed that may provide more accurate surveillance data.[70, 71] Programs must be able to detect and identify hidden and difficult to control larval sites (e.g., cryptic locations such as septic tanks, storm drains, sump pumps, and vacant lots) and other productive sites, as well as the readily identified and commonly found larval habitats.

2. Personal Protection

Individuals may reduce the likelihood of infection by the use of personal repellents on skin or clothing. DEET (N,N-diethyl-m-toluamide) and picaridin (also known as KBR3023 or Bayrepel™) are effective repellents widely available in the Americas. Infants and others sleeping or resting during the day should use bednets to avoid infection from *Ae. aegypti* and *Ae. albopictus*, both of which are day biting mosquitoes. It is of particular importance that individuals who are

potentially infected with CHIKV during an outbreak rest beneath an IT bednet to avoid mosquito bites and further spread of infection. Use of IT bednets has the additional benefit of killing mosquitoes that come into contact with the net, which may reduce vector-human contact for other household members.[72] A number of pesticide products may be used to safely treat bednets (Table 6), or long-lasting pretreated nets can be obtained commercially.

Table 6. **WHO recommended insecticide products for treatment of mosquito nets.**[a]

1. Conventional treatment:		
Insecticide	Formulation[b]	Dosage[c]
Alpha-cypermethrin	SC 10%	20–40
Cyfluthrin	EW 5%	50
Deltamethrin	SC 1%; WT 25%; and WT 25% + Binder[d]	15–25
Etofenprox	EW 10%	200
Lambda-cyhalothrin	CS 2.5%	10–15
Permethrin	EC 10%	200–500

2. Long-lasting treatment:		
Product name	Product type	Status of WHO recommendation
ICON® MAXX	Lambda-cyhalothrin 10% CS + Binder Target dose of 50 mg/m²	Interim

[a]Adapted from http://www.who.int/whopes/Insecticides_ITN_Malaria_ok3.pdf

[b]EC = emulsifiable concentrate; EW = emulsion, oil in water; CS = capsule suspension; SC= suspension concentrate; WT = water dispersible tablet

[c]Milligrams of active ingredient per square meter of netting.

[d]K-O TAB 1-2-3

3. Household Prevention

The use of intact screens on windows and doors will reduce entry of vectors into the home, and mosquito proofing water storage vessels will reduce oviposition sites and local production. Within a household, use of IT bednets[72] and IT curtains[73] also reduce vector-human contact.

The number of adult mosquitoes in a home may be reduced by using commercially available pyrethroid-based aerosol sprays and other products designed for the home, such as mosquito coils and electronic mat vaporizers. Aerosol sprays may be applied throughout the home, but areas where adult mosquitoes rest (dark, cooler areas) must be targeted, including bedrooms, closets, clothing hampers, etc. Care should be taken to emphasize proper use of these products when advocating their application to the public, in order to reduce unnecessary exposure to pesticides.

4. Neighborhood and Community Prevention

Neighborhood and community prevention for a CHIKV introduction in the Americas should be based on methods developed for dengue control, utilizing effective strategies to reduce the densities of vector mosquitoes.[66] A fully operational dengue control program will reduce the probability that a viremic human arriving in the Americas will be fed upon by ***Ae. aegypti*** or ***Ae. albopictus*** mosquitoes, thereby leading to secondary transmission and potential establishment of the virus.

Dengue programs for controlling ***Ae. aegypti*** have traditionally focused on control of immature mosquitoes, often through the community's involvement in environmental management and source reduction. It is essential that community involvement be incorporated into an IVM program.[74, 75]

Vector Control Procedures

The WHO Dengue Guidelines[66] provide information on the main methods of vector control, and they should be consulted when establishing or improving existing programs. The program should be managed by experienced professional vector control biologists to assure that the program uses current pesticide recommendations, incorporates new methods of vector control, and includes resistance testing. Prevention programs should utilize the methods of vector control found in Appendix F, as appropriate.[66, 74]

7B. Response to CHIKV Introduction

Immediately upon confirmation of the first autochthonous CHIKV case, the health department should inform the IVM program regarding the onset date and location of the case. Vector control procedures must be intensified to effectively reduce the abundance of infected vectors in order to halt transmission in the areas of the case(s).

Simultaneously, emergency response committees at local and national levels should be informed of the situation and activated. Initial efforts should focus on containing virus transmission and preventing expansion (Appendix G). If virus containment fails, or if cases are not detected until the outbreak has spread over a large geographic area, intensive vector control efforts will need to be expanded to a larger scale program.

Summary of Vector Surveillance and Control Section

- Epidemiological surveillance is key to the timely detection of cases Early detection of disease will increase the likelihood of containing transmission of CHIKV in the area.
- Successful IVM for CHIKV requires trained experts in medical entomology and vector control, sufficient resources, and a sustained commitment.
- Current dengue control programs in the Region should be utilized and improved to prevent CHIKV transmission.
- Vector surveillance and control activities and methodologies must be validated and continually evaluated to measure efficacy.

8. RISK AND OUTBREAK COMMUNICATION

8A. Risk Communication for CHIKV Introduction or Outbreaks

Effective communication to the community and various stakeholders is crucial to avoid confusion and misinformation and to engage people in steps to reduce the risk of disease. Under IHR, risk communication for public health emergencies includes the range of communication capabilities through the preparedness, response, and recovery phases of an outbreak.[55] Messages should encourage informed decision making, positive behavior change, and the maintenance of trust in public authorities. As CHIKV is new to the Americas, the media, the public, and many officials will need to be educated about the disease, its mode of transmission, the lack of specific therapeutic treatment, means of symptomatic and supportive treatment, and the adoption of control measures. Risk communication messaging can emphasize that the risk of CHIKV infection can be reduced, and that it is typically a self-limiting disease.

8B. Risk Communication Strategies by Phase and Target Audience

Appendix H gives an example of a model risk communication plan with strategies organized by preparedness, response, and recovery phases of an emergency. The plan defines various target audiences that should be considered in developing a country-specific risk communication plan.

Risk communication should be organized across multiple agencies and should target the media, the public health community, community-based organizations, the private sector, and civil society institutions.

Structure and Coordination

Ideally, an emergency response to a CHIKV outbreak will use an Incident Command System that provides structure for collaboration. In Latin America, the equivalent is the Emergency Operational Committee (or COE in Spanish). A key component in emergency operations is the establishment of a Joint Information Center (JIC) that allows for coordination of messages from local, state, national, and international partners. Information about setting up and running a JIC can be found online at: http://www.fema.gov/emergency/nims/PublicInformation.shtm

As part of the emergency operations structure, communication staff should work closely with other operational components (epidemiology, vector control, etc.). All groups should meet regularly ensure that they are in agreement on key data points, including number of cases, geographic factors, and messages. Lack of coordination on these points will help create confusion and undermine confidence in the management of the response.

Strategies by Phase: Preparedness Phase

The primary activities during the preparedness phase are to develop a communications plan and to create strategic partnerships. During this phase, potential activities may include:

- Informing key stakeholders about preparedness materials, such as these guidelines.
- Developing basic response materials, such as fact sheets and frequently asked questions, will facilitate a rapid response to a CHIKV introduction and reduce misinformation. Information channels may include printed materials, websites and other electronic and social media, the mass media, short message service (SMS) text messages, inter-personal communication through group meetings, schools, and utilization of traditional or folk media.
- Working with partners to develop strategies to guide care seeking, travel (national and international), and prevention/risk reduction.
- Communication with journalists and news agencies to provide baseline information on CHIKV and on the national preparedness and response plan.
- Networking with key personnel at potential information points, such as arrivals and departure locations (airports, ports, borders) and public facilities (health care facilities, educational centers, workplaces, nursing homes, shopping malls, churches, public transport sites, stadiums, among others).
- Anticipating sensitive issues can allow for preemptive preparation of responses and strategies. Sensitive topics related to CHIKV may include concern over safety of community and household pesticide use, any restrictions involved in a containment response, large numbers of persons seeking care at health care facilities, and the cost of control measures.

Strategies by Phase: The Response Phase

During the response phase, the communication plan is put into action; in particular, communications with the mass media, health care providers, and other key audiences are intensified.

The Mass Media

Effective communication through the mass media can help maintain clear information regarding the outbreak and the public health response. Information should be communicated via an appropriate, trained national-level spokesperson. Use of a consistent spokesperson can build trust and avoid the release of potentially conflicting messages from various sources. Information in the mass media can also reinforce the key behavioral outcomes that can help reduce risk during an outbreak. Content in the electronic and print media should be regularly monitored (on a daily basis during an intense outbreak), in order to make any necessary adjustments to the strategies and messages conveyed to the population.

Response to media inquiries should be timely and accurate, and should include promotion and prevention issues. Messaging for media responses should be coordinated through the JIC. Sensitive issues should be addressed promptly and transparently, following best crisis and risk communication principles: http://www.bt.cdc.gov/cerc/

It is useful to employ multiple channels to disseminate accurate information on the disease and its prevention. These may include advertising and other social markering tools (e.g., TV, radio, printed media, the Web, outdoor billboards, and social networks, such as Twitter, Facebook, or YouTube). Relying on multiple channels may be especially important when the outbreak engenders confusion and controversy.

Health Care Provider Communication

Because CHIKV is a new disease in the Region, many health care providers probably will have little specific information available on diagnosis and care for CHIK patients. Mechanisms for rapid communication with care providers should be established, such as dedicated health care provider websites, health alert network notices, and communication via professional associations. Ideally, basic materials can be prepared in advance of an outbreak. Specific communication strategies should reflect the actual availability of electronic media to health care providers throughout the Region. See Appendix H for further details.

Strategies by Phase: The Recovery Phase

During the recovery phase, the main activities include guiding the general population on the sustainment of appropriate public health measures, and informing the public when the risk of disease transmission has been reduced. At this point there also is an opportunity to evaluate and assess the effectiveness of risk communication efforts. A summary evaluation at the end of the outbreak will provide valuable insight for future responses. For further details refer to Appendix H.

8C. Specific Behavioral Strategies for CHIKV Risk Reduction

Specific strategies for effective personal, household, and community primary prevention are discussed in section 7 (Vector Surveillance and Control). Messages regarding control measures should be developed in collaboration with vector control staff, and should emphasize specific steps that households must consider to optimize potential control measures (e.g., leaving windows open during fogging, which materials to remove from the home in the event of indoor residual application, what the larvicide looks like and how long to leave in place, etc.).

Advance research into knowledge, attitudes, and practices regarding repellent and household control measures may yield benefits in understanding barriers to use and potential for misapplication. Even if it is not feasible to conduct this research in advance, rapid qualitative assessment in affected areas can yield insights to increase the effectiveness of prevention messages.

Communication on prevention should target specific behaviors that offer the best likelihood of reducing risk. Feasible strategies will vary by location, depending on a given community's resources, attitudes, control program capacity, and ecology. Key messages for personal and household prevenrion can include:

Community Strategies

- Encouraging support of and compliance with governmental control efforts such as environmental sanitation, larviciding and adulticiding.
- Advocacy for household and neighborhood source reduction (e.g., trash clean-up, control of water storage, etc.).

Household and Personal Strategies

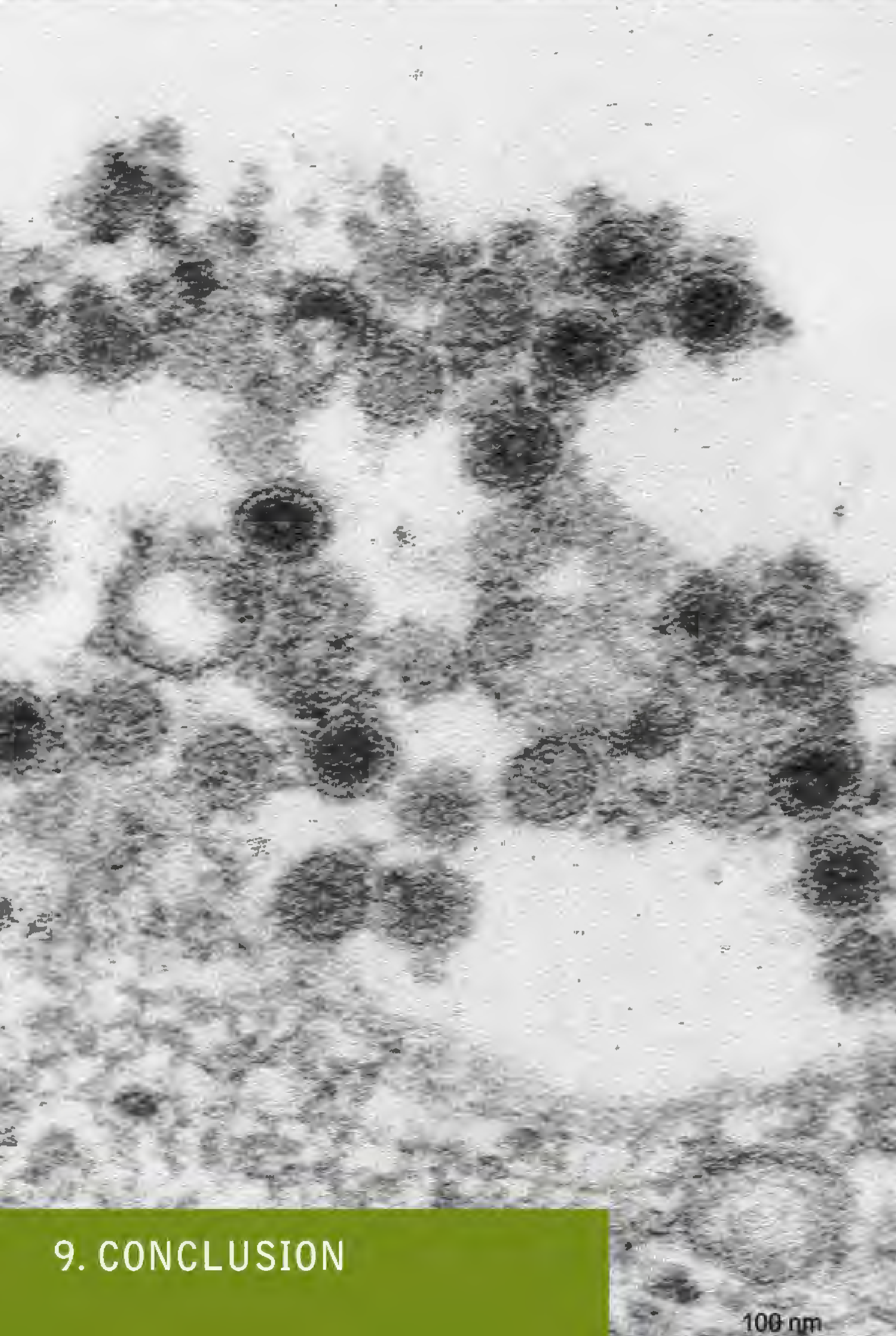
- Use of personal prevention such as clothing, repellents, and insecticide-treated materials:
  - Encouraging the use of long sleeves and pants may be reasonable in areas where temperatures are moderate, particularly during evening when *Aedes* mosquitoes are often still seeking a bloodmeal. This recommendation may be less practical in tropical zones.
  - Repellents for use on skin and clothing are now sold widely throughout the Americas. A significant outbreak may increase interest in these tools, and authorities should be prepared to provide guidance and to rely on creative strategies to increase use.

- Methods to reduce human-vector contact include use of household insecticides and installation of screening. Where feasible, screening material can be installed over windows even without the use of expensive frames (stapling in place or using wooden frames).

- It may be useful to specify active ingredients or even brand names for recommended repellent and/or household insecticides, as a way to reduce use of ineffective and possibly dangerous materials.

Summary of Risk and Outbreak Communication Section

- Communications are an integrated, coordinated effort involving all disciplines and components for preparation and response.
- Timely communication with stakeholders is crucial for enlisting the community's participation and to avoid confusion and misinformation.
- As CHIKV is novel in the Americas, the media, the public and many officials will need to be educated about the disease, the mode of transmission, the lack of specific therapeutic treatment, means of symptomatic and supportive treatment, and effective control measures.



9. CONCLUSION

Currently, CHIKV is not known to be circulating in the Americas. The risk of introduction is high, however, due to travel importation, competent vectors (same vectors as dengue), and population susceptibility. Given the likelihood that CHIKV will be introduced in the Region, advance preparation is essential. The timely detection of cases and an appropriate and rapid response with active participation of all stakeholders will be necessary to minimize the risk of importation and sustained transmission in the Region.

These guidelines for the preparedness and response CHIKV introduction in the Americas were developed to increase awareness of the disease and to provide the necessary information to institute the most appropriate strategies to prevent the importation and spread of CHIKV in the Region. Each Member Country is encouraged to use and adapt these guidelines to detect an outbreak of the disease early, to conduct pertinent epidemiologic investigations, and to prevent or mitigate the expansion of the disease in the Americas.

10. APPENDICES

Appendix A. Viral Isolation Protocol (for Cell Culture)

Introduction	The optimal method for determining specific etiology of an arbovirus infection requires isolation of the virus from a specimen obtained from the patient during the acute stage of the disease and the demonstration of a rise in titer of an antibody to the isolate during convalescence. For a number of reasons, the successful isolation of most arboviruses from patient specimens is the exception, whether because the specimen to be examined is not collected early enough, is not properly handled, or is not expeditiously transmitted to the virus laboratory for inoculation. The viremia for many arbovirus infections in humans, if detectable at any stage, ceases by the time of or soon after onset of symptoms a stage when antibody is often demonstrable. Because some circulating virus may be recoverable and the antibody may be absent, or present in low titer, the acute-phase blood specimen should be collected immediately upon suspicion of a viral etiology. Delay of an hour or so can compromise the chance of virus isolation; the allowable time depends upon the type of viruses involved. Certain arboviruses, like CHIKV, produce a viremia of sufficient magnitude and duration that the viruses can be isolated from blood during the acute phase of illness, e.g., 0 to 5 days after onset. Viral isolates can be recovered by biopsy or at autopsy from the viscera of patients with acute disease.

Introduction	For isolation from brain, samples should be taken from several areas, including the cortex, brain nuclei, cerebellum, and brain stem. Neurotropic arboviruses sometimes can be isolated from CSF obtained by lumbar puncture during the acute stages of encephalitis or aseptic meningitis. Alphaviruses, like CHIKV, have been isolated from joint fluid of patients with acute polyarthritis. Under certain circumstances arboviruses have been recovered from urine, milk, semen, and vitreous fluid.
Principle	Susceptible cell culture systems are available for the attempted isolation of the presumed etiologic agent of an illness or disease. Following successful isolation, the isolate may be positively identified and an antigen prepared from this isolate or the virus itself may be used to test the patient's serum for the presence of antibodies to the viral isolate. If antibodies are detected, this exercise confirms that the isolate was the causative agent of the illness or disease. In certain instances, serum from a patient may not be available. Under those circumstances, one relies on reisolation of the causative virus from the same original specimen. Reisolation should always be attempted, however, whether serum is available from the patient or not.
Materials and Reagents	Vero cell culture monolayers or other suitably susceptible cell cultures C6/36 cell cultures-cloned *Ae. albopictus* mosquito cell.
Procedure	Available tissues or fluids should be divided for viral isolation, electron microscopy, and for immunohistochemical examination. Tissues should be collected aseptically and rapidly transported to the laboratory in viral transport. The aliquot for viral isolation should be immediately frozen at –70° C in a mechanical freezer or stored on dry ice. Samples for viral isolation should be kept frozen continuously, avoiding freeze-thaw cycles that inactivate virus.

(Continued)

Appendix A. Viral Isolation Protocol (for Cell Culture) (Cont.)

Procedure	The aliquot for electron microscopy should be minced and place directly in glutaraldehyde. Autolytic changes occur rapidly and tissues should be fixed as quickly as possible. A portion of the sample should be fixed in buffered formalin or, preferably, embedded in freeze-media and frozen, to prepare sections for immunohistochemical examination. Processed specimens should be inoculated into cell cultures with a minimum of delay. Sera from patients with acute febrile illnesses can be used undiluted for virus isolation or at dilutions of 1:10 and 1:100 in a protein containing diluent. It is important to inoculate unknown specimens at two or preferably more dilutions (undiluted to 10^{-2}). Shell vial cultures or $25cm^2$ flask cultures of Vero are inoculated and observed for the production of CPE during 10–14 days. For shell vials, a total volume of 400 µl is inoculated, followed by centrifugation at 100 x g for one hour at 37°C. A portion of the cell supernatant can be collected and tested for the presence of virus by either targeted RT-PCR or consensus RT-PCR assays. Alternatively cells are harvested and spot slides are prepared for IFA examination using monoclonal dengue type-specific antibodies.
Controls	Uninoculated Vero and C6/36 cells
Interpretation	Positive virus isolation, reisolation, and definitive identification define the etiologic agent of the patient's illness. If paired sera or a convalescent serum from that patient are available, the identified viral isolate is tested serologically with the patient's sera to verify antibody response to that virus.

Bibliography

- Tsai, TH: Arboviruses, p.606-618. In Rose NR, de Marcario EC, Fahey JL, Friedman H, and Penn GM (eds): Manual of Clinical Laboratory Immunology, 4th ed. American Society for Microbiology, Washington, DC, 1992.
- Karabatsos N: Arboviruses. Chap. 27. In Hsiung GD, Fong C, Landry M (eds): Diagnostic virology, 4th ed, Yale University Press, New Haven, CT, 1993.
- Schmidt NJ: Cell culture techniques for diagnostic virology. In Lennette EH, Schmidt NJ (eds): Diagnostic procedures for viral, rickettsial, and chlamydial infections, 5th ed, American Public Health Association, Washington, DC 1979.
- Beaty BJ, Calisher CH, and Shope RS: Arboviruses, p. 797-856. In Schmidt NJ, Emmons RW (eds): Diagnostic procedures for viral, rickettsial and chlamydial infections, 6th ed, American Public Health Association, Washington, DC, 1989.

Appendix B. Real-Time Reverse Transcriptase-Polymerase Chain Reaction Protocol

Real time RT-PCR can be performed using a number of commercially available kits. Either the BioRad iScript 1 Step RT-qPCR (#170-8895) or the QIAGEN QuantiTect Probe RT-PCR kit (#204443) is currently used by the Arboviral Diagnostic Laboratory at DVBD, CDC. The two kits are nearly identical in the reaction setup, with one exception: the volume of enzyme used in the QIAGEN kit is 0.5 µl per reaction, instead of 1.0 µl; the volume of water in the master mix is adjusted by 0.5 µl to account for this. The setup shown below is for the QIAGEN kit. Note also that the volume of RNA added per reaction below is 10 µl but can be increased or decreased with the appropriate adjustment of total volume with water.

Component	Vol. per reaction	10 Reactions
RNase free water	13.2 µl	132 µl
2X Ready mix	25 µl	250 µl
primer 1 (100 µM stock)	0.5 µl	5 µl
primer 2 (100 µM stock)	0.5 µl	0.5 µl
FAM/ probe (25 µM stock)	0.30 µl	3.0 µl
enzyme	0.5 µl	5 µl

Prepare a reagent "master mix" according to the number of reactions desired. The master mix should be prepared in a "clean room" that is physically separated from all other laboratory activities and that has dedicated reagents and equipment (e.g., pipettes). For 10 samples make a 10X master mix (see above) by multiplying the volumes of all individual reagents by 10. Combine the reagents in the above order in an RNase free centrifuge tube **on ice**. Divide the master mix into 10 portions of 40 µl each into either 0.2 ml optical (specifically for TaqMan assays; emission fluorescence is read through the cap) PCR tubes or a 96 well optical PCR plate. Finally add 10 µl of the individual RNA sample to each tube or well. All samples are tested in duplicate wells. **Include several "NO RNA"** negative controls (NTC) by adding water instead of any RNA. Include a positive control or a dilution series of known quantities of positive control RNA if setting up a quantitative assay.

Cycling conditions (QIAGEN conditions for Real Time RT-PCR):		
1 cycle:		**45 cycles:**
50°C for 30 min	(RT reaction)	95°C 15 sec
95°C for 15 min	(enzyme activation)	60°C 1 min

(Continued)

Appendix B. Real-Time Reverse Transcriptase-Polymerase Chain Reaction Protocol (Cont.)

Interpretation

The following algorithm is used by the Arboviral Diagnostic Laboratory at DVBD, CDC to evaluate the TaqMan results.

Positive:	• Cutoff (Ct) value 38 in duplicate wells
Equivocal:	• Ct value 38 in one of two wells
Negative:	• Ct values >38 in duplicate wells

All positive and equivocal samples are repeated with a second set of primer/probes for confirmation. A positive result in any of the negative controls invalidates the entire run. Failure of the positive control to generate a positive result also invalidatas the entire run.

I. RNA EXTRACTION

Avoiding Contamination and Working with RNA

- Maintain physically separated work areas; one should be dedicated to **pre-amplification RNA work** (RNA extraction and RNA addition) and the other to **Master mix** production.
- Utilize dedicated or separate equipment within pre- and post-amplification areas, especially pipets and centrifuges.
- Always wear gloves, even when handling unopened tubes.
- Quickly open and close tubes and avoid touching any inside portion.
- Use RNase free plastic disposable tubes and pipet tips.
- Use aerosol block pipet tips.
- Use RNase free water.
- Prepare all reagents on ice.

1. Solid phase samples (mosquitoes or tissues) are first homogenized in an isotonic buffer to produce a liquid homogenate. RNA is extracted from liquid specimens (CSF or serum) without any pre-treatment as described below. Tissue specimens (~10mm^3) are homogenized in 1 ml of BA-1 diluent using TenBroeck tissue grinders. Mosquito specimens are homogenized in TenBroeck tissue grinders or by using the copper clad steel bead (BB) grinding technique. With both techniques the homogenates are clarified by centrifugation in a microcentrifuge (e.g., Eppendorf) at maximum speed for 5 minutes to pellet any particulate material.

(Continued)

2. Extract RNA from 140 µl of the liquid specimen (CSF, serum, or clarified homogenate) using the QiAmp viral RNA kit (QIAGEN part # 52904). Follow the manufacturer's protocol exactly. **NOTE: For mosquito specimens add one additional wash with AW1**. Extract at least two negative controls and two positive controls along with the test specimens. The positive controls should differ in the amount of target RNA present (i.e., a pre-determined high positive and a low positive). The volume of sample extracted can be greater or less than the standard volume stated in the QIAGEN protocol (140 µl) with the appropriate adjustments to all other volumes in the protocol.

Appendix C. IgM and IgG Serologic Assay Protocols

IgM ANTIBODY CAPTURE ENZYME-LINKED IMMUNOSORBENT ASSAY PROTOCOL

Introduction	Assays that detect viral specific immunoglobulin M (IgM) are advantageous because they detect antibodies produced during the first few days after onset of clinical symptoms in a primary infection. This obviates the need for convalescent-phase specimens in many cases. IgM capture is the optimal approach to IgM detection: it is simple, sensitive, and is applicable to serum and cerebrospinal fluid (CSF) samples from a variety of animal species (e.g., human, equine, avian). False-positive reactions due to rheumatoid factor are minimized.
Principle	IgM antibody capture enzyme-linked immunosorbent assay (MAC-ELISA) provides a useful alternative to immunofluorescence for documentation of a serologic response. ELISA is less subjective than immunofluorescence and large numbers of samples can be processed. The principle of ELISA is similar to that of immunofluorescence. Anti-IgM (the capture antibody) is coated on 96-well plates in the Arboviral Diagnostic Laboratory at DVBD, CDC. This is followed sequentially by the patient's serum, then known non-infectious viral antigen. The presence of antigen is detected by using enzyme-conjugated anti-viral antibody, and a colorimetric result is generated by the interaction of the enzyme and a chromogenic substrate. This constitutes the MAC-ELISA.
Safety	The procedure should be performed under laboratory safety conditions that take into consideration the potential infectious nature of the serum specimens involved. Lab coat, gloves, and a laminar flow hood is recommended.

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IgM ANTIBODY CAPTURE ENZYME-LINKED IMMUNOSORBENT ASSAY PROTOCOL

Materials and Reagents	**Coating buffer:** Carbonate/bicarbonate buffer pH 9.6, 1.59g Na_2CO_3 + 2.93g $NaHCO_3$ diluted in 1L water. **Wash buffer:** Phosphate buffered saline (PBS), 0.05% Tween 20, pH 7.2. PBS is available in powdered form from multiple commercial sources. **Blocking buffer:** PBS/5% milk/ 0.5% Tween 20. **Stop solution:** 1 N H_2SO_4. **Coating antibody:** Goat anti-human IgM, Kirkegaard and Perry Laboratories cat# 01-10-03. **Viral antigen:** Sucrose-acetone extracted suckling mouse brain viral antigens, non-infectious, previously titrated. **Normal antigen:** Sucrose-acetone extracted suckling mouse brain antigen from mock-infected animals. **Detecting antibody conjugate:** Horseradish peroxidase conjugated monoclonal antibody, previously titered. **Substrate:** 3,3'5, 5' tetramethylbenzidine base (TMB-ELISA), Gibco cat# 15980-0414. **Plates:** Immulon II HB flat-bottomed 96 well plates, Dynatech Technologies cat# 3455. Microplate washer Microplate reader

Materials and Reagents	Incubator Single and multi-channel pipettors Reagent reservoirs Ziploc bags, paper towels
Clinical specimens	Acute and convalescent human serum and/or cerebrospinal fluid (CSF) specimens Previously tested antibody-positive and antibody-negative human sera for controls Note: Store all diagnostic specimens at 4°C prior to testing, and -20°C after all anticipated testing has been completed. Avoid repeated freeze-thaw cycles.
Procedure	Note: The following procedure includes information on quality control and interpretation. Each serum specimen is tested in triplicate on both viral and normal antigens. Eight test specimens can be analyzed per plate. CSF specimens are usually tested only singly. 1. Using a fine-tipped permanent marker, number and label the plates. Identify the location of each clinical specimen (S1–S8) by using the appropriate laboratory code number. *To keep timing of reagent addition consistent, process plates in the order that they are numbered during all steps of the procedure.* Plates should be kept in an enclosed, humidified environment during all incubation times with the exception of the coating step. A large ziploc bag containing a moist paper towel works well for this purpose.

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IgM ANTIBODY CAPTURE ENZYME-LINKED IMMUNOSORBENT ASSAY PROTOCOL

Procedure	2. Coat the inner 60 wells of 96 well plates with 75 µl well of goat anti-human IgM diluted 1:2000 in coating buffer pH 9.6. **Incubate at 4°C overnight.** 3. Dump out the coating antibody and blot plates on paper towels. Block plates with 200µl blocking buffer per well. **Incubate at room temperature for 30 minutes.** 4. Wash wells 5X with wash buffer by using an automatic plate washer. Wells should be filled to the top each cycle. 5. Add 50µl per well of the patient's serum (S) diluted 1:400 in wash buffer to a block of six wells, or add patient's CSF undiluted to two wells only, so that the CSF will be tested singly against the viral and normal antigens. Note: CSF can be diluted to a maximum of 1:5 in wash buffer if necessary. Add positive control human serum (Ref) diluted in wash buffer according to a previous titration, and a negative human serum control (N) diluted 1:400 in wash buffer to a block of six wells each. Incubate plates for **1 hour at 37°C** in a humidified chamber. 6. Wash 5X. 7. Dilute viral antigen in wash buffer according to a previous titration. Add 50µl per well to the left three wells of each serum block. To the right three wells of each block, add 50µl per well of normal antigen diluted in wash buffer to the same concentration as the viral antigen. **Incubate overnight at 4°C** in a humidified chamber. 8. Wash 5X.

Procedure

9. Add 50µl per well of horseradish peroxidase-conjugated monoclonal antibody, broadly cross-reactive for the appropriate viral antigenic group, diluted in blocking buffer, according to a previous titration. **Incubate for one hour at 37°C in a humid chamber**.
10. Turn on plate reader to warm up, and remove TMB-ELISA from refrigerator.
11. Wash plates 5X **twice**. Turn the plates 180° in the washer after the first series of five cycles. This promotes consistent results.
12. While the plate is at room temperature, add 75µl per well of TMB substrate to all wells. Immediately cover plates to block out light. Incubate at room temperature for **10 minutes**. A blue color will develop in antibody-positive wells.
13. Add 50µl per well of stop solution to all wells, including the outer rows of wells on the plate (the plate reader itself should be set to zero on some of these wells). The wells that were blue will now change to a yellow color. Allow plates to sit at room temperature for one minute. Read plates in microtiter plate reader by using a 450 nm filter.

(Continued)

Appendix C. IgM and IgG Serologic Assay Protocols (Cont.)

IgM ANTIBODY CAPTURE ENZYME-LINKED IMMUNOSORBENT ASSAY PROTOCOL

Practical considerations	1. Plates can be coated and kept at 4°C for up to a week. 2. Undiluted control sera can be stored at 4°C for up to two weeks. 3. Reconstituted, undiluted viral and normal antigens can be stored at –20°C for an undefined period of time. 4. Test and control sera can be diluted to the working dilutions and refrigerated one day prior to use. Antigens and conjugate *must be diluted to the working dilutions immediately prior to use.* The MAC-ELISA should be restandardized periodically. This should occur when new lot numbers of reagents are introduced, and at the very least, once a year. It is recommended that the mean optical density of the positive control serum reacted on the viral antigen be set to approximately at 1.0. The normal control serum reacted on the viral antigen should be around 0.2 (this varies). The standardization of reagents is normally achieved via titration, always comparing the optical densities of the reagents when reacted on viral and normal antigen.
Results	Before the results can be calculated for each clinical specimen, the test must be determined to be **valid**. For a **valid** test the following must be true:

Results	$\frac{\text{Mean OD of the positive control serum reacted on viral antigen (P)}}{\text{Mean OD of the negative control serum reacted on viral antigen (N)}}$ must be greater than or equal to 2.0. **This is the P/N of the positive control.** Test validity must be determined for each plate. Results for the clinical specimens may only be determined if the test is valid. If the test is not valid, then that plate must be repeated. If the test still fails after a repeat, then one or more of the reagent or test parameters was likely in error, and troubleshooting should be performed. To determine whether the clinical specimens (S1–S8) contain IgM to the viral antigen (which would indicate recent infections with that virus) the following must be calculated: $\frac{\text{Mean OD of the test specimen reacted on viral antigen (P)}}{\text{Mean OD of the negative control serum reacted on viral antigen (N)}}$ **This is the P/N of the test specimen.** For a specimen to be considered IgM-positive to the test virus, the P/N must be greater than or equal to 2.0. **In addition** the value of P for the test specimen must be greater than or equal to twice the mean OD of the test specimen reacted on normal antigen. If this requirement is not met, non-specific background is being generated, and the result **must** be reported as uninterpretable.

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IgM ANTIBODY CAPTURE ENZYME-LINKED IMMUNOSORBENT ASSAY PROTOCOL

Interpretation	All patient P/N values greater than or equal to 2.0 should be reported as presumptive IgM-positive (see paragraph below), as long as they meet the requirements listed above. In the event that an early acute CSF or serum is negative by this test, a convalescent serum specimen must be requested and tested before that patient is reported as negative for serological evidence of recent viral infection. Without testing of a convalescent specimen, a negative result may reflect testing of an acute-phase specimen obtained before antibody has risen to detectable levels. In most patients, IgM is detectable eight days post-onset of symptoms from an alpha-, flavi-, or California group virus infection. IgM persists for at least 45 days, and often for as long as 90 days. The positive P/N cut-off value of 2.0 is empirical, based on experience and convention. P/N values that lie between 2.0 and 3.0 should be considered suspect false-positives. Further tests should be performed to determine the status of these specimens. It should be stressed that the P/N value for a specimen at the screening dilution of 1:400 is not an indication of absolute antibody concentration, i.e., the P/N value is not quantitative.

Interpretation	It is further recommended that for sera, all positive results should be confirmed by titration using 6, 2-fold dilutions of the serum specimens compared to a similar titration of the negative control serum. Linear curves indicate true seropositivity. Flat or undulating titration curves indicate false-positive results.
References	• Tsai, TH: Arboviruses, In Rose NR, Marcario EC, Fahey JL, Friedman H, and Penn GM, (Eds): Manual of Clinical Laboratory Immunology, 4th Edition, American Society for Microbiology: 606-618, 1976. • Diagnosis of Infections caused by Viruses, Chlamydia, Rickettsia, In Koneman EW, Allen SD, Janda WM, Schreckenberger PC, and Winn Jr. WC , (Eds): Diagnostic Microbiology, 4th Edition, JB Lippicott Co: 956-1074, 1992. • Monath, TP, Nystrom, RR, Bailey, RE, Calisher, CH, and Muth, DJ:Immunoglobulin M antibody capture enzyme-linked immunosorbent assay for diagnosis of St. Louis encephalitis. J Clin Microbiol. 1984;20:784-790. • Martin, DA., Muth, DA., Brown, T., Karabatsos, N., and Roehrig, JT. Standardization of immunoglobulin M capture enzyme-linked immunosorbent assays (MAC-ELISA) for routine diagnosis of arboviral infections. J Clin Microbiol. 2000 May; 38(5): 1823-6.

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IgG ENZYME-LINKED IMMUNOSORBENT ASSAY PROTOCOL

Introduction	Immunoglobulin G (IgG) is less virus-specific than IgM, appears in serum slightly later in the course of infection than IgM, and remains detectable until long after IgM ceases to be present. Using the IgG-ELISA in parallel with the IgM Antibody Capture Enzyme-linked immunosorbent assay (MAC-ELISA), one can observe the relative rises and falls in antibody levels in paired serum samples. The test is simple and sensitive. It is applicable to serum specimens but not generally to CSF samples. False-positive reactions due to rheumatoid factor are minimized.
Principle	The IgG-ELISA provides a useful alternative to immunofluorescence for identification of a viral isolate or documentation of a serologic response. IgG-ELISA is less subjective than immunofluorescence and large numbers of samples can be processed. Viral group-reactive monoclonal antibody is coated on a 96-well plate, followed sequentially by known viral antigen, patient serum, enzyme-conjugated human IgG, and lastly substrate for the conjugate used. This constitutes the IgG-ELISA used at the Arboviral Diagnostic Laboratory, DVBD, CDC.

Safety	The procedure should be performed under laboratory safety conditions that take into consideration the potential infectious nature of the serum specimens involved. Lab coat, gloves, and a laminar flow hood is recommended.
Materials and Reagents	**Coating buffer:** Carbonate/bicarbonate buffer pH 9.6, 1.59g Na_2CO_3 + 2.93g $NaHCO_3$ diluted in 1L water. **Wash buffer:** Phosphate buffered saline (PBS), 0.05% Tween 20, pH 7.2. PBS is available in powdered form from multiple commercial sources. **Blocking buffer:** 3% goat serum, 1% Tween-20, in PBS. **Coating antibody:** Group-specific monoclonal antibody, previously titrated. **Viral antigen:** Sucrose-acetone extracted suckling mouse brain viral antigens, non-infectious, previously titrated. **Normal antigen:** Sucrose-acetone extracted suckling mouse brain antigen from mock-infected animals. **Detecting antibody conjugate:** Alkaline phosphatase-conjugated goat anti-human IgG Fcγ portion, previously. titrated (Jackson Immunoresearch cat# 109-055-098) **Substrate:** 3 mg/ml p-nitrophenyl phosphate, disodium (Sigma 104, Sigma diagnostics cat# 104-105) in 1M Tris (base) pH 8.0 (note: the Tris requires considerable conc. HCl for the pH adjustment). **Stop solution:** 3M NaOH.

(Continued)

IgG ENZYME-LINKED IMMUNOSORBENT ASSAY PROTOCOL	
Materials and Reagents	**Plates:** Immulon II HB flat-bottomed 96 well plates. Dynatech Technologies cat# 3455. Microplate washer Microplate reader Incubator Single and multi-channel pipettors Reagent reservoirs Ziploc bags, paper towels
Clinical specimens	Acute and convalescent human serum Note: Store all diagnostic specimens at 4°C prior to testing, and at -20°C after all anticipated testing has been completed. Avoid repeated freeze-thaw cycles.

Procedure	Note: The following procedure includes information on quality control and interpretation. Each serum specimen is tested in triplicate on both viral and normal antigens. Eight test specimens can be analyzed per plate. 1. Using a fine-tipped permanent marker, number and label the plates. Identify the location of each clinical specimen (S1–S8) by using the appropriate laboratory code number. To *keep timing of reagent addition consistent, process plates in the order that they are numbered during all steps of the procedure.* Plates should be kept in an enclosed, humidified environment during all incubation times with the exception of the coating step. A large ziploc bag containing a moist paper towel works well for this purpose. 2. Coat the inner 60 wells of 96 well plates with 75µl/well of the appropriate group-reactive monoclonal antibody diluted in coating buffer according to prior titration. **Incubate at 4°C overnight.** 3. Dump out the coating antibody and blot plates on paper towels. Block plates with 200µl blocking buffer per well. **Incubate at room temperature for 30 minutes.** 4. Wash wells 5X with wash buffer by using an automatic plate washer. Wells should be filled to the top each cycle. 5. Dilute viral antigen in wash buffer according to a previous titration. Add 50µl per well to the left three wells of each serum block. To the right three wells of each block, add 50µl per well of normal antigen diluted in wash buffer to the same concentration as the viral antigen. **Incubate overnight at 4°C** in a humidified chamber. 6. Wash 5X. 7. Add 50µl per well of the patient's serum (S) diluted 1:400 in wash buffer to a block of six wells. Add positive control human serum (Ref) diluted in wash buffer according to a previous titration, and a negative human serum control (N) diluted 1:400 in wash buffer to a block of 6 wells each. **Incubate plates for one hour at 37°C** in a humidified chamber.

(Continued)

IgG ENZYME-LINKED IMMUNOSORBENT ASSAY PROTOCOL

Procedure	8. Wash 5X. 9. Add 50µl per well of alkaline phosphatase-conjugated goat anti-human IgG diluted in blocking buffer, according to prior titration. **Incubate for one hour at 37°C** in a humid chamber. 10. Turn on plate reader to warm up and dissolve substrate tablets in tris buffer about 15 minutes prior to adding it to the plates. 11. Wash plates 5X **twice**. Turn the plates 180° in the washer after the first series of five cycles. This promotes consistent results. 12. While the plate is at room temperature, add 75µl per well of Sigma 104 substrate to all wells. Immediately cover plates to block out light. Incubate at room temperature for **30 minutes.** A yellow color will develop in antibody-positive wells. 13. Add 35µl per well of stop solution to all wells, including the outer rows of wells on the plate (the plate reader itself should be set to zero on some of these wells). Reactive wells will remain a yellow color. Allow plates to sit at room temperature for one minute. Read plates in microtiter plate reader by using a 405 nm filter.

Practical considerations	1. Plates can be coated and kept at 4°C for up to a week. 2. Undiluted control sera can be stored at 4°C for up to two weeks. 3. Reconstituted, undiluted viral and normal antigens can be stored at –20°C for an undefined period of time. 4. Test and control sera can be diluted to the working dilutions and refrigerated one day prior to use. Antigens and conjugate **must be diluted to the working dilutions immediately prior to use.** The IgG-ELISA should be restandardized periodically. This should occur when new lot numbers of reagents are introduced, and at the very least, once a year. It is recommended that the mean optical density of the positive control serum reacted on the viral antigen be set to approximately. 1.0. The normal control serum reacted on the viral antigen should be around 0.2 (this varies). The standardization of reagents is normally achieved via titration, always comparing the optical densities of the reagents when reacted on viral and normal antigen.
Results	Before the results can be calculated for each clinical specimen, the test must be determined to be valid. For a valid test the following must be true: $\frac{\text{Mean OD of the positive control serum reacted on viral antigen (P)}}{\text{Mean OD of the negative control serum reacted on viral antigen (N)}}$ must be greater than or equal to 2.0. **This is the P/N of the positive control.**

(Continued)

IgG ENZYME-LINKED IMMUNOSORBENT ASSAY PROTOCOL

Results	Test validity must be determined for each plate. Results for the clinical specimens may only be determined if the test is valid. If the test is not valid, then that plate must be repeated. If the test still fails after a repeat, then one or more of the reagent or test parameters was likely in error, and troubleshooting should be performed. To determine whether the clinical specimens (S1-S8) contain IgG to the viral antigen (which would indicate either recent or past infections with that virus) the following must be calculated: $\frac{\text{Mean OD of the test specimen reacted on viral antigen (P)}}{\text{Mean OD of the negative control serum reacted on viral antigen (N)}}$ **This is the P/N of the test specimen.** For a specimen to be considered IgG-positive to the test virus, the P/N must be greater than or equal to 2.0. In addition the value of P for the test specimen **must be** greater than or equal to twice the mean OD of the test specimen reacted on normal antigen. If this requirement is not met, non-specific background is being generated, and the result must be reported as uninterpretable.

Interpretation	All patient P/N values greater than or equal to 2.0 should be reported as presumptive IgG-positive (see the explanatory paragraph on the following page), as long as they meet the requirements listed above. **Interpretations of IgG-ELISAs should always be made in the context of the corresponding MAC-ELISA, and the date of collection with respect to onset of symptoms.** A positive IgG-ELISA result on its own cannot distinguish a recent from a past infection, due to the persistence of IgG from past infections. IgG is also more cross-reactive than IgM, which means that a positive result by the IgG-ELISA may in fact indicate the presence of antibody to a related virus. In most cases, IgG is detectable 12 days post-onset of symptoms from an alpha-, flavi-, or California group virus infection and persists for long periods of time, possibly for years. Some examples of common scenarios are listed below: 1. A positive IgG-ELISA result with a positive MAC-ELISA result would indicate the presence of a recent infection. 2. A negative IgG-ELISA result with a positive MAC-ELISA result in an acute specimen would indicate a recent infection in which the IgG antibody had not yet risen to detectable levels. 3. A positive IgG-ELISA result and a negative MAC-ELISA result from a specimen timed between approximately 8 and 45 days post-onset of symptoms would suggest the occurrence of a past infection (remember that IgG to a virus is often cross-reactive with other viruses from the same genus).

(Continued)

Appendix C. IgM and IgG Serologic Assay Protocols (Cont.)

IgG ENZYME-LINKED IMMUNOSORBENT ASSAY PROTOCOL

Interpretation	4. For a single late specimen (obtained later than 45 days post-onset of symptoms) yielding a positive IgG-ELISA result and a negative MAC-ELISA result, the distinction between the current infection and past infections cannot be made. 5. A negative IgG-ELISA result plus a negative MAC-ELISA result indicates the lack of any recent or past infections with the test virus if the sample was collected >7 days post illness onset. These results on a more acute sample cannot rule out the infection as the antibody response may not have had time to form. The positive P/N cut-off value of 2.0 is empirical, based on experience and convention. P/N values that lie between 2.0 and 3.0 should be considered suspect false-positives. Further tests should be performed to determine the status of these specimens. It should be stressed that the P/N value for a specimen at the screening dilution of 1:400 is not an indication of absolute antibody concentration, i.e., the P/N value is not quantitative.

References	
	• Tsai, TH: Arboviruses, In Rose NR, Marcario EC, Fahey JL, Friedman H, and Penn GM, (Eds): Manual of Clinical Laboratory Immunology, 4th Edition, American Society for Microbiology: 606-618, 1976. • Diagnosis of Infections caused by Viruses, Chlamydia, Rickettsia, In Koneman EW, Allen SD, Janda WM, Schreckenberger PC, and Winn Jr. WC , (Eds): Diagnostic Microbiology, 4th Edition, JB Lippicott Co: 956-1074, 1992. • Johnson, AJ., Martin, DA., Karabatsos, N., and Roehrig, JT. Detection of anti-arboviral immunoglobulin G by using a monoclonal antibody-based capture enzyme-linked immunosorbent assay. J Clin Microbiol. 2000 May; 38(5): 1827-31.

Appendix D. Example of a Case Report Form

Basic Data

Last name ______________________ First name: ______________________

Sex: ( ) male ( ) female

Date of birth: ____/____/____/ age |__|__| years |__|__| months |__|__| days

Occupation: ______________________

Address: ______________________

Zipcode: |__|__|__|__|__|__|__|__| telephone number: |__|__|__|__|__|__|__|

Clinical Information

Clinical history number: ______________________

Date of symptom onset: ____/____/____/ Epidemiological week: |__|__|

Number of days with symptoms: ____/____/ Date of first medical consult: ____/____/____/

Date of hospitalization: ____/____/____/

Death: Yes ( ) No ( ) Date: ____/____/____/

Symptoms

	Yes	No
Fever	☐	☐
Arthritis	☐	☐
If yes, where		
Hands	☐	☐
Feet	☐	☐
Ankles	☐	☐
Other	☐	☐
Arthralgia	☐	☐
Periarticular edema	☐	☐
Skin manifestations	☐	☐

	Yes	No
Myalgia	☐	☐
Back pain	☐	☐
Headache	☐	☐
Nausea	☐	☐
Mucosal bleeding	☐	☐
Vomiting	☐	☐
Asthenia	☐	☐
Meningoencephalitis	☐	☐

If yes, describe ______________________

Other ______________________

Clinical diagnosis ______________________

Laboratory information

Blood sample testing for CHIKV infection:

Date of collection: ____/____/____/

Serology - IgM	Yes	☐	No	☐		
Result:	Positive	☐	Negative	☐	Date of result	____/____/____/
Serology - IgG	Yes	☐	No	☐		
Result:	Positive	☐	Negative	☐	Date of result	____/____/____/
RT-PCR	Yes	☐	No	☐		
Result:	Positive	☐	Negative	☐	Date of result	____/____/____/
Viral isolation	Yes	☐	No	☐		
Result	Positive	☐	Negative	☐	Date of result	____/____/____/

(Continued)

Appendix D. Example of a Case Report Form (Cont.)

Epidemiological information

History of travel within the previous 30 days prior to symptom onset: Yes No
If yes, where: Country ________________ City ______________
Place of residence:
Community ________________________________ Locality ____________________________

Blood or blood products received within the previous 30 days prior to symptoms onset
Yes No

Final classification:

Discarded: |__|
Confirmed: |__|
Suspected: |__|
Date of notification: _____/_____/_____/

Name of reporting personnel: __________________________

Appendix E. Report for an Event or Outbreak of Public Health Importance

NOTIFICATION____________Region_____________

A case or outbreak of *[EVENT OF HEALTH]* has occurred in the town *[LOCATION]*, commune, region [NAME Municipalities and Regions] on *[MONTH and YEAR or time period]*.

As of *[Report Date]*, *[CASE NUMBER]* of *[EVENT OF HEALTH]* presenting with *[MAIN SIGNS AND SYMPTOMS]* were seen at *[INSTITUTION OR SECTOR OR OTHER COMMUNITY]*. These cases are occurring in an areas with an approximate population of *[N ° OF INHABITANTS or exposed population]*.

The cases have occurred between the *[START DATE, WEEK EPIDEMIOLOGICAL]* and *[DATE TO END* or *TODAY]*. The affected area is mainly [urban or rural] and has previously presented occasional outbreaks of *[PREVIOUS OUTBREAKS]*.

The most striking feature of the cases is *[FEATURE PERSON: SEX, AGE, OR OTHER TO DEFINE CHARACTERISTICS of people affected]*.

Of all the cases, *[# DECEASED]* died and *[# HOSPITALIZED]* requiring hospitalization. Cases either dying or requiring hospitalization have *[COURSE TYPE: DEATH, HIGH WITHOUT COMPLICATIONS, AFTERMATH, ETC]*.

[# samples] sample(s) of *[SPECIMEN TYPE]* have been taken and sent to [LAB] for processing where testing is ongoing or has confirmed [Etiologic agent].

Epidemiological research indicates that the outbreak was produced by *[POSSIBLE MECHANISM, SOURCE, exposure factor]*.

The control actions have been taken are as follows: *[ACTION]*

Note: The immediate notification must include whatever is possible to complete the learning of the outbreak. Once investigated, it is sent back the full format to regional World Health Organization office.

Appendix F. Vector Control Procedures

There are a number of vector control procedures that should be considered to mitigate the risk of CHIKV expansion in an area (Table F1).

Table F1. Vector control procedures

Environmental management	Adult mosquito control
• Reduce larval habitats • Manage (wash/cover) containers • Discard/recycle containers • Reduce human-vector contact • Install window screens	• Use of IT bednets • Use of IT curtains • Lethal ovitraps • Space sprays • Indoor residual treatments
Larval control • Source reduction • Chemical control • Biological control	**Resistance testing** **Operational research and efficacy evaluation**

Chemical Control of Larval Habitats

If potable water vessels cannot be screened or covered, they should be cleaned regularly or treated to stop larval production according to WHO Pesticide Evaluation Scheme (WHOPES) recommended practices for potable water.[66] Potential larval habitats that do not contain water intended for human consumption may be treated with larvicides listed in Table F2.

Table F2. WHO-recommended compounds and formulations for control of mosquito larvae in container habitats.[a]

Insecticide	Formulation[b]	Dosage[c]	WHO hazard classification of active ingredient[d]
Organophosphates			
Pirimiphos-methyl	EC	1	III
Temephos	EC,GR	1	U
Insect growth regulators			
Diflubenzuron	DT,GR, WP	0.02-0.25	U
rs-methoprene[e]	EC	1	U
Novaluron	EC	0.01-0.05	NA
Pyriproxyfen[e]	GR	0.01	U
Biopesticides			
Bacillus thuringiensis[e] israelenses	WG	1-5 mg/L	U
Spinosad	DT,GR,SC	0.1-0.5	U

[a] WHO recommendations on the use of pesticides in public health are valid only if linked to WHO specifications for their quality control. WHO specifications for public health pesticides are available at http://www.who.int/whopes/quality/en/. Instructions must always be followed when using insecticides.

[b] DT=tablet for direct application; GR=granule; EC=emulsifiable concentrate; WG=water-dispersible granule; WP=wettable power; SC=suspension concentrate.

[c] mg/L of active ingredient for control of container-breeding mosquitoes.

[d] Class II=moderately hazardous; Class III=slightly hazardous; Class U=Unlikely to pose an acute hazard in normal use; NA=not available.

[e] Can be used at recommended dosages in potable water.

Space Sprays for Adult Mosquito Control

Space sprays for *Ae. aegypti* and *Ae. albopictus* control are most effective when the inside of houses and associated yards are individually treated with handheld sprayers. Repeated applications are required to kill newly emerging adults. In an epidemic response, space sprays should be carried out with handheld sprayers whenever possible, or with truck-mounted sprayers to increase speed of coverage, every two to three days.[66] Attention to resistance testing, calibration of equipment, droplet size, and timing of application are all critical to effective use of these tools.[66] Large-scale truck and airplane based application of pesticides is generally not effective in controlling *Ae. aegypti* when used alone.[76] Large-scale space spraying must be used as a component of an IVM program to be effective. Table F3 provides information on insecticides suitable to *Ae. aegypti* and *Ae. albopictus* control.

Table F3. Examples of insecticides for cold aerosol or thermal fog application against mosquitoes.[a]

Insecticide	Chemical	Dosage of active ingredient (g/ha)		WHO hazard classification of active ingredient[c]
		Cold aerosols	Thermal fogs[b]	
Fenitrothion	Organophosphate	250–300	250–300	II
Malathion	Organophosphate	112–600	500–600	III
Pirimiphos -methyl	Organophosphate	230–330	180–200	III
Bioresmethrin	Pyrethroid	5	10	U
Cyfluthrin	Pyrethroid	1–2	1–2	II
Cypermethrin	Pyrethroid	1–3	–	II
Cyphenothrin	Pyrethroid	2–5	5–10	II
d,d-trans-Cyphenothrin	Pyrethroid	1–2	2.5–5	NA
Deltamethrin	Pyrethroid	0.5–1.0	0.5–1.0	II
D-Phenothrin	Pyrethroid	5–20	-	U
Etofenprox	Pyrethroid	10–20	10–20	U
λ Cyhalothrin	Pyrethroid	1.0	1	II
Permethrin	Pyrethroid	5	10	II
Resmethrin	Pyrethroid	2–4	4	III

[a] Adapted from: Pesticides and their application for the control of vectors and pests of public health importance.[77] Label instructions must always be followed when using insecticides.

[b] The strength of the finished formulation when applied depends on the performance of the spraying equipment used.

[c] Class II=moderately hazardous; class III=slightly hazardous; class U=unlikely to pose an acute hazard in normal use; NA=not available.

Indoor Residual Sprays for Adult Mosquito Control

Traditionally, Indoor Residual Sprays (IRS) have been used most successfully against malaria vectors (Table F4). IRS treatment should be effective against *Ae. aegypti*, which rests indoors, though it may be difficult to apply operationally. Generally, all interior walls and ceilings of a house are treated. For control of *Ae. aegypti*, it is important to treat bedrooms, closets, the undersides of beds, and other dark areas where *Ae. aegypti* adults rest before and after taking a bloodmeal. Residents should be informed that IRS are safe when applied according to the label, but that individuals with health concerns, such as those with asthma or allergies, should take measures to reduce or eliminate exposure during the application process.

Resistance Testing

Frequent application of the same insecticide or class of insecticide may select for individual mosquitoes that are able to survive pesticide applications.[78] Resistance is a heritable change in the sensitivity of a mosquito population to an insecticide that may lead to failure of the pesticide to yield the expected degree of control.

The insecticides available for use as adulticides are limited, and fall into three chemical classes: organophosphates, carbamates, and pyrethroids. Some products for larviciding have different modes of actions, such as insect growth regulators and microbials tools.[78] However, the most commonly used product for controlling larvae of *Ae. aegypti* in containers is the organophosphate temephos. Resistance to temephos has been detected in multiple *Ae. aegypti* populations in the Americas[79, 80] and poses a serious threat to *Ae. aegypti* control. Little information is available about resistance in *Ae. albopictus* populations in the Region.

Control programs must include a resistance monitoring program[81-83] (additional references are available at http://www.who.int/whopes/resistance/en/) to assess efficacy and to establish a pesticide rotation plan to mitigate the development of resistance.

Table F4. WHO recommended insecticides for use as indoor residual sprays.[a]

Insecticide compounds and formulations[b]	Class group[c]	Dosage g a.i./m²	Mode of action	Duration of effective action (months)
DDT WP	OC	1–2	contact	>6
Malathion WP	OP	2	contact	2-3
Fenitrothion WP	OP	2	contact & airborne	3-6
Pirimiphos-methyl WP & EC	OP	1–2	contact & airborne	2-3
Bendiocarb WP	C	0.1–0.4	contact & airborne	2-6
Propoxur WP	C	1–2	contact & airborne	3-6
Alpha-cupermethrin WP & SC	PY	0.02–0.03	contact	4-6
Bifenthrin WP	PY	0.025–0.05	contact	3-6
Cyfluthrin WP	PY	0.02–0.05	contact	3-6
Deltamethrin WP, WG	PY	0.02–0.025	contact	3-6
Etofenprox WP	PY	0.1–0.3	contact	3-6
Lambda-cyhalothrin WP, CS	PY	0.02–0.03	contact	3-6

[a] Available at (http://www.who.int/whopes/Insecticides_IRS_Malaria_09.pdf).

[b] CS = capsule suspension; EC = emulsifiable concentrate; SC = suspension concentrate; WG = water dispersible granule; WP = wettable

[c] OC = Organochlorines; OP = Organophosphates; C = Carbamates; PY = Pyrethroids

Note: WHO recommendations on the use of pesticides in public health are valid only if linked to WHO specifications for their quality control. WHO specifications for public health pesticides are available on WHO's homepage at http://www.who.int/whopes/quality/en/.

Supervision, Safety, and Quality Assurance

Continuous monitoring and supervision are required to ensure that staff are adequately trained and are following appropriately technical guidelines for pesticide application and personal safety.[77] IVM programs must include a quality assurance program designed to monitor the effectiveness of the control activities. A quality assurance program should monitor applicator performance and control outcomes. Control failures may be due to misapplication, incomplete coverage, or insecticide resistance, and must be corrected immediately. Quality assurance efforts should be continuous, systematic, and independent.

Appendix G. Vector Control for CHIKV Containment

Virus containment efforts should be initiated upon discovery of a CHIKV case or cluster (introduced or autochthonous transmission), simultaneous with activating the local emergency response capacity. The purpose of containment is to eliminate the newly introduced CHIKV and to prevent its spread by implementation of intensive vector control measures. This concept has been applied to contain the invasion and spread of dengue viruses in non-endemic areas.[84] Even if CHIKV spreads into a country's urban area, containment should be considered a primary strategy to avoid its spread elsewhere in that country and into neighboring countries. Application of vector control measures should start at homes of detected CHIKV cases (or at a suspected site of infection) and should be applied to the entire neighborhood. Because of delays in case detection and notification, it is likely that CHIKV may have already spread to other parts of the neighborhood.[85] Request the involvement of local authorities to gain access to closed or abandoned properties. The entire emergency containment operation needs to be conducted rapidly, so human and other resources devoted to this effort should be matched to the size of the containment area. Malaria control personnel and others with suitable training may be utilized to accomplish goals of the containment effort. The following actions are recommended to contain an introduction of CHIKV:

1. In addition to participating in a national communication effort, immediately inform the community (residents, schools, churches, businesses, etc.) of the CHIKV introduction. Topics should include mode of spread, symptoms, advice to consult a physician if symptoms appear, and community involvement to eliminate standing water from containers and to allow health inspectors into homes for application of anti-mosquito measures. Prepare the community so that CHIKV containment operations can be conducted more efficiently and rapidly in residential and commercial properties, as well as in public spaces and parks.

2. Conduct indoor and outdoor insecticide applications to eliminate adult mosquitoes. Details on insecticides, dosages, and precautions can be found in Table F3 and in WHO publications.[66, 77, 86]

3. Simultaneously conduct container elimination/protection and larviciding to eliminate the production of new mosquitoes. Special attention should be given to cryptic or subterranean bodies of water that can produce *Aedes* mosquitoes, such as roof gutters, drains, wells, elevated water tanks, water meters, and even septic tanks.[87] Water storage containers and animal drinking pans should be cleaned (by scrubbing and rinsing) and protected with tight covers. Some containers, such as useful implements (paint trays, buckets) and bottles should be stored in a way to prevent them from collecting water (e.g., upside-down, under a roof). Large objects that accumulate rain water (boats, cars) should be properly covered. Containers that cannot be prevented from holding water for any reason should be treated with a larvicide. For example, containers holding water for animal or human consumption require the application of larvicides that have been licensed in the country for that particular purpose. WHO's approved larvicides used to treat potable water-storage containers are provided in Appendix F, in the section "Chemical control of larval habitats". Pesticides should always be used following their label specifications. For other larvicides that can be applied to containers holding non-potable water, see Table F2.[66, 77, 86, 88]

4. Alternatively, or concurrently with source reduction, residual insecticides can be applied to containers holding non-potable water (to inner/outer walls) to kill the larvae and pupae and to nearby outdoor surfaces to kill landing or resting adult mosquitoes. This type of insecticide application is done with hand-held compression sprayers and much care has to be taken to avoid spraying near unprotected water-storage containers or pets.[66, 77]

5. Monitor houses and buildings in the neighborhoods that are being treated and implement special control rounds after working hours, weekends, and holidays to assure that nearly 100% of homes and businesses are treated.

Outbreak Intervention

Controlling an epidemic of CHIKV or a series of outbreaks over a larger geographic scale requires the following:

1. Activating a command center (Emergency Operations Center), either physical or virtual, where epidemiologists, entomologists and vector control specialists, educators, media communicators, etc., can jointly plan, work, and evaluate progress throughout the epidemic. Epidemiological services need to be organized so that daily, detailed reports are sent to all authorized personnel in the affected areas (states, municipalities). To be successful, it will be necessary to establish an efficient system of communications, allowing for feed-back reports and the receipt of acknowledgements (by e-mail, fax, telephone, etc.).

2. Orienting the population at large through the media on the possibility of resulting infection with CHIKV and on how families and communities can contribute to the abatement of the epidemic. Educational materials on specific actions to prevent or control CHIKV transmission should be elaborated and distributed by various media (TV, radio, newspapers, local organizations, schools, clinics, etc.). It is important to report daily (to the press) which communities or neighborhoods are being affected by CHIKV, so that residents and local authorities are aware of imminent risk of infection and can take appropriate actions (e.g., proper use of repellents, elimination of all standing water, organizing clean-up campaigns, etc.). Dissemination of this information needs to be done in a way that no personal information or identifiers are released to the public at any time.

3. Ensuring that infected and febrile persons are protected from mosquito bites by using bednets at home and in hospitals.

4. Orienting vector control operations through real-time epidemiological and entomological assessments of CHIKV transmission, indicating the specific areas that need to be treated. In areas where dengue is endemic,

knowledge from a retrospective analysis of dengue virus transmission or previous experience with dengue viruses should be used to guide vector control operations.

5. Applying effective vector control measures. An epidemic is generally a series of smaller outbreaks occurring simultaneously in several different places within a country (neighborhoods, cities, municipalities, states), where the number of disease cases is unusually large. This means that epidemic control measures may need to be applied concurrently in several locations. Large-area control of mosquito populations over short periods by spraying insecticides from truck- or aircraft-mounted equipment has not proven effective in reducing dengue transmission. Large-scale outdoor application of pesticides may be beneficial when used in conjunction with other control measures as part of an integrated mosquito control program.[76] Therefore, effective vector control measures to be applied during an epidemic are similar to those recommended for area-wide CHIKV containment (above) and dengue virus outbreaks.[86] The main difference is that they should be simultaneously applied in many areas to abate individual outbreaks.

    a. Geo-reference each CHIKV case to the level of operational control areas. In the case of endemic areas, conduct the retrospective epidemiological study at this level, so that stratification serves operational purposes. Use Geographical Information System (GIS) to map operational units, make and distribute maps of disease incidence, and spatially monitor the epidemic.
    b. Divide the target area (e.g., state, municipality) into relatively uniform areas (operational control areas) that will be treated using an area-wide approach (neighborhoods with 2,000–5,000 persons; census areas, zip-codes, etc.). All premises, businesses and other areas (parks, cemeteries, abandoned lots, areas along creeks, illegal dumps, etc.) will be simultaneously treated within a few days. This operational division of the space should be conducted well in advance of an eventual introduction of CHIKV.

c. Area-wide vector control measures imply having sufficiently trained personnel, equipment, and supplies to treat the environment where *Aedes* mosquitoes are being produced. By significantly reducing mosquito adults (using adulticides) and the production of new adult mosquitoes (source reduction and elimination, larvicides) in a particular area, the transmission cycle could be interrupted, and CHIKV could be driven to extinction. This scenario is possible only if the number of biting mosquitoes is dramatically reduced for the length of time it takes for humans and vectors to become clear of CHIKV. For this reason, vector control measures need to achieve a very high efficiency, as measured by the elimination of an extremely large proportion of vector mosquitoes.

Limitations of Vector Control

Vector population reduction and the associated reduction of vector-human contact should be correlated with reduced virus transmission and reduced human disease. In order to interrupt an outbreak, however, vector population reduction must be immediate, substantial, and sustained. Adult mosquitoes will continue to emerge and replace adult mosquitoes killed by adulticides. Therefore, it is essential to maintain IVM programs with complete coverage and repeated treatments. In addition to the presence of mosquito control professionals and an active IVM program, it is important to maintain the support and cooperation of all members of society.[67]

Appendix H. Model of Risk and Outbreak Communication Plan

Target audience	Preparation phase	Response phase	Recovery phase
Government authorities	• Prepare briefing to authorities on the risk of introduction of CHIKV, in coordination with subject matter experts. • Train spokespersons on this subject. • Develop a plan for risk and crisis communication. • Coordinate with the media and other social stakeholders.	• Activation of communication plan.	• Evaluation and adjustment of communication plan.

(Continued)

Appendix H. Model of Risk and Outbreak Communication Plan (Cont.)

Target audience	Preparation phase	Response phase	Recovery phase
Public health and emergency response authorities	• Establish protocol for use of incident management/ emergency operations approach if not in place. • Conduct exercises to allow communication responders to know emergency response structure and their roles.	• Establish JIC within Emergency Operations Center. • Establish regular meetings of Public Information Officers (PIOs) and strategic communication staff for all agencies involved and regular meeting schedule with other key elements of operational response.	• Conduct "lessons learned" assessment of communications response and use of emergency response structure.

Medical personnel	• Develop and provide information via websites, booklets, pamphlets, and pocket guides. • Participate in conferences addressing risk factors, case presentations, diagnostics, and risk factors. • Develop frequently addressed questions (FAQs) addressing differences between CHIKV and dengue, if applicable. • Establish hotline infrastructure for clinical support.	• Implement response plan(s). • Provide updated, easy-to-access information concerning epidemiology of outbreak, risk factors, case definition, diagnostics, etc. • Update information flow as necessary. • Activate and staff an information hotline for clinical support.	• Continue to provide updates. • Continue to support the clinical hotline. • Provide information concerning sequelae. • Evaluate communication with the clinical community; gather "lessons learned". • Provide final response report.

(Continued)

Appendix H. Model of Risk and Outbreak Communication Plan (Cont.)

Target audience	Preparation phase	Response phase	Recovery phase
Hospitals	• Develop and provide information for preparedness planning, patient management. • Develop a handbook or pocket guide addressing the type of information that should be shared with CHIKV patients, patients' families, hospital personnel, and hospital-associated personnel (emergency medical personnel).	• Implement contingency plans with hospitals. • Gather information from the hospitals to support information and counseling for CHIKV patients, patients' families, hospital personnel, and associated personnel (emergency medical personnel, Red Cross, paramedics, fire services, public safety, etc.).	• Evaluate the communications plan. • Gather information for "lessons learned". • Provide final report to the hospital community.

Hospitals		• Use gathered information to facilitate communications with other sectors and the general population concerning the status of hospital operations and medical care support locations.	

(Continued)

Appendix H. Model of Risk and Outbreak Communication Plan (Cont.)

Target audience	Preparation phase	Response phase	Recovery phase
Associations of health professionals and medical sciences	• Collaborate with associations to educate members via lectures, newsletters, social networking, and websites to address risk factors, case definition and diagnostics, treatment, and sequelae. • Provide the associations with FAQ sheets. • Work with associations to provide prevention messages to the general population.	• Intensify communication with the medical sciences and health-professional associations with respect to health care services and look for disease patterns and trends.	• Evaluate the timeliness of information provided to the associations, as well as the timeliness of transfer of the information to the association's membership.

Laboratory – government and private laboratories	• Develop and provide information addressing sample management, tests, procedures, and materials in both electronic and hardcopy formats via video conferences, workshops, etc.	• Activate information channels for the timely gathering of information to support the decision cycles at the operational level, including the health care services.	• Evaluate communications with the laboratory system. • Continue to gather information from laboratories. • Gather "lessons learned".

(Continued)

Appendix H. Model of Risk and Outbreak Communication Plan (Cont.)

Target audience	Preparation phase	Response phase	Recovery phase
Vector control personnel	• Vector control personnel and communicators work together to develop and provide information concerning possible CHIKV vectors and integrated vector management, in both electronic and hardcopy formats via video conferences, workshops, etc.	• Activate communication plan with health professionals and other entities. • Gather information concerning the effectiveness of ongoing integrated vector management activities, if appropriate. • Provide updated information to health professionals concerning protection and prevention.	• Evaluate communications actions for vector control and gather "lessons learned". • Gather information concerning best practices for vector management.

Local and regional health department personnel; epidemiologists	• Health department staff, epidemiologists, and communicators work together to develop and provide information to be used by public health partners and the media to address the surveillance methods, analysis of data, and development of messages for the general population.	• Activate information channels for the timely gathering of information to support decision cycles at the operational level, including the health care services.	• Evaluate communications with health departments and epidemiologists. • Gather "lessons learned".

(Continued)

Appendix H. Model of Risk and Outbreak Communication Plan (Cont.)

Target audience	Preparation phase	Response phase	Recovery phase
Blood banks	• Provide information to blood bank managers concerning risks associated with CHIKV. • Develop and provide information concerning blood-product management and risks, as well as preparation for donor shortages. • Develop donor screening guidelines and procedures. • Develop fact sheets for donors and prospective donors for distribution in the blood banks.	• Establish active communication with blood banks to address shortages of supplies and of donors within restricted areas, in order to inform the general population as well as the media. • Coordinate with implementation of donor screening guidelines and procedures in areas affected by CHIKV emergence.	• Evaluate the effectiveness of recommendations that blood banks provide to blood donors. • Develop a communications plan to support lifting of restrictions for donations within a previously restricted area.

Travelers associations, businesses, and organizations	• Outreach to those traveling to regions at risk for CHIKV, describing symptoms and prevention of the disease, using official and business websites and factsheets, and other means (such as closed circuit TV, message boards, and public service announcements).	• Request travel and tourism industry operators to intensify the communication activities included in the travelers information plan. • Provide updates concerning disease status and preventive and protective actions.	• Evaluate the timeliness of response by the travel industry. • Gather "lessons learned".
Maritime, land, and air transportation industry and authorities (ports)	• Develop THANs before the event for use by port authorities, customs and transportation security agencies. • Provide the industry and authorities with information concerning IHR requirements.	• Request maritime, land, and air industry and port representatives to intensify their communications activities as appropriate for the response. • Provide updates concerning disease status and preventive and protective actions.	• Evaluate the timeliness of response by the travel industry. • Gather "lessons learned".

(Continued)

Appendix H. Model of Risk and Outbreak Communication Plan (Cont.)

Target audience	Preparation phase	Response phase	Recovery phase
Civil authorities, government officials	• Engage in advocacy to gain the support needed for effective preparation and response. • Keep channels open with local, regional, and national levels of government. • Designate and train spokespersons, providing function-specific information appropriate for the level of responsibility.	• Implement the communications plan with the other government authorities, updating spokespersons information. • Include appropriate representatives in the JIC.	• Evaluate the effectiveness of preparation and response communications activities conducted with authorities and officials. • Gather "lessons learned".

General population	• Use multiple channels to inform the general public of the potential for CHIKV risk and means of prevention and protection. • Plan for use of hotlines; support local hotlines as appropriate. • Develop health education materials, such as website pages, posters, pamphlets, handbills, billboards, SMS text messaging and social media, and on-line social networking. • Consider the use of interpersonal communication through group meetings, in schools; make optimal use of traditional/folk media.	• Special campaigns may be carried out via the mass media, including in local newspapers/ magazines, radio, and TV, as well as through outdoor publicity, such as billboards. • Monitor communication channels. Assess delivery of the messages. • Increase efforts to garner support of insecticide use and other control measures, as needed. • Develop location-specific messaging and update as appropriate. • Open hotlines, and support local hotlines as appropriate.	

(Continued)

Appendix H. Model of Risk and Outbreak Communication Plan (Cont.)

Target audience	Preparation phase	Response phase	Recovery phase
The media	• Develop and maintain relationships with the media that will support communications activities. • Provide training, participate in interviews, and develop public service announcements to advice media partners and prepare them for potential CHIKV activity. • Prepare spokespersons. Spokespersons must be technically and politically credible and willing to interact with the press on short notice.	• Establish a permanent channel of information with the media for regular communications, including briefings and interviews. • Disseminate regular reports from the JIC concerning the status of outbreak to provide a consistent message. • Monitor press reports and coverage. Conduct analysis of reports for appropriateness and relevance and adjust messaging/ strategies accordingly.	• Continue to provide updates to the media, including appropriate messaging as the risk of transmission is reduced. • Evaluate implementation of the communications plan to introduce necessary adjustments to it. • Gather "lessons learned".

Faith-based communities	• Develop and provide information for use within religious media networks, during services, and among outreach groups.	• Collaborate with leadership to advance protection and prevention efforts and vector management.	• Evaluate involvement in the communications plan for the preparation and response to CHIKV.
Nongovernmental organizations (NGOs), humanitarian groups, community-based health organizations, and other civil society organizations	• Collaborate with these organizations on outreach to organize, sensitize, and educate their communities.	• Collaborate with leadership to advance protection and prevention efforts and vector management.	• Evaluate involvement in the communications plan for the preparation and response to CHIKV.

(Continued)

Appendix H. Model of Risk and Outbreak Communication Plan (Cont.)

Target audience	Preparation phase	Response phase	Recovery phase
Educational system	• Collaborate with the educational system to develop lessons, teaching materials, and content that will raise awareness of CHIKV, as well as sanitation and other preventive measures. • Seek to have lessons on CHIKV risks and response in the school curriculum, as a way to promote and expand awareness; the students will become communications multipliers.	• Collaborate with leadership to advance protection and prevention efforts and vector management.	• Evaluate involvement in the communications plan for the preparation and response to CHIKV.

Private sector, business	• Collaborate with the private sector in preparing its plan to organize, sensitize, and educate their organizations, employees, and customers. • Seek to involve the private sector in the government's efforts in communications activities for preparation and prevention.	• Collaborate with the private sector to intensify its communications activities and to further the government`s communication initiatives addressing protection and prevention efforts and vector management. • Provide updates to the private sector concerning the response.	• Evaluate involvement of private sector in the communication plan for preparation and response to CHIKV. • Gather "lessons learned".

Appendix I. Meeting of the Technical Advisory Group of Preparedness and Response for Chikungunya Virus Introduction in the Americas

Objectives

The aim of this meeting was to assemble a technical advisory group to review and adapt a preliminary draft of "Preparedness and Response for Chikungunya Virus Introduction in the Americas". The technical advisory group included experts in various fields from the Americas, including epidemiologists, clinicians, entomologists, laboratory personnel, and communication specialists. After discussing the document's various chapters, these experts submitted changes, additions, and rewrites that they considered appropriate to make the guidelines factual and relevant to all countries in the Region. The guidelines are meant to be a useful tool that can be adapted and applied by each Member Country in establishing the most appropriate strategies for the prevention and control of Chikungunya virus in the Americas.

Agenda

Wednesday, 7/21/10

8:30 – 9:00	Reception for participants
9:00 – 9:30	Welcome and presentation of the meeting's objectives and working dynamics CDC (Roger Nasci) and PAHO/WHO (Otavio Oliva, Luz Maria Vilca)
9:30 – 10:15	Chikungunya Virus: Clinical and Epidemiological aspects. CDC (Ann Powers)
	Break
10:45 – 11:30	Laboratory Diagnosis of Chikungunya virus: An overview CDC (Robert Lanciotti)

11:30–12:30 Impact of a Chikungunya outbreak on public health: Experience of La Réunion

FRANCE, Laveran Military Hospital (Fabrice Simon)

Lunch

13:30–14:30 Chikungunya cases identified in the Americas: USA, Canada, and French Territories

CDC (Erin Staples)

CANADA, National Microbiology Laboratory, WHOCC (Michael Drebot)

FRENCH TERRITORIES (Philippe Quenel)

14:30 – 15:15 Round table: Control of *Aedes aegypti* in the Americas: what has worked and what has not

CDC (Roberto Barrera)

BRAZIL Ministry of Health (Irma Braga)

CDC (Harry Savage)

PAHO/WHO (José Luis San Martín)

PAHO/WHO (Chris Frederickson)

Break

15:45 – 16:15 Assignment of work groups and review of goals for groups (five working groups were formed for reviewing the draft guideline "Preparedness and Response for Chikungunya Virus Introduction in the Americas"):

- Epidemiological Aspects (Epidemiology, Surveillance, and Outbreak Response chapters)
- Clinical aspects (Clinical, Case Management chapters)
- Laboratory (Laboratory Chapter)
- Entomology (Vector Surveillance and Control chapters)
- Communications (Communications Chapter)

16:15 – 17:45 Groups meet to decide on approach (Coordinator, Presenter)

Thursday, 7/22/10

8:30 – 10:00	Working groups (Cont.)
	Break
10:30 – 12:30	Working groups (Cont.)
	Lunch
13:30 – 15:00	Working groups (Cont.)
	Break
15:30 – 17:00	Make changes to draft guidelines (edit manuscript) and meet to draft presentations of proposed changes

Friday, 7/23/10

8:30 – 09:00	Groups meet to finalize draft presentation of proposed changes
	Break
9:30 – 11:30	Group presentations
11:30 – 12:30	Group presentations
	Lunch
13.30 – 14.30	Additional changes to the draft guideline (final edits to manuscript)
	Break
15.00 – 16.00	Wrap-up and next steps CDC (Roger Nasci) and PAHO/WHO (Otavio Oliva)

List of Participants

Pan American Health Organization:

Dr. Otavio Oliva (HSD/IR/V)

Dr. José Luis San Martín (HSD/IR/D)

Dr. Luz Ma. Vilca (HSD/IR/V)

Ms. Olivia Brathwaite (PWR/PAN)

Ms. Vivien Lawis (HSD/IR/V)

Participants by Working Group:

A. Epidemiological surveillance:

Dr. Andrea Olea, Chile (Ministry of Health)

Dr. Diana Patricia Rojas, Colombia (Ministry of Health, Instituto Nacional de Salud)

Dr. Yeni Herrera, Peru (Ministry of Health)

Dr. Philippe Quenel, Martinique. (CIRE, Institut de Veille Sanitaire)

Dr. Joel Montgomery, Peru (NMRCD)

Dr. Luz Maria Vilca, USA (PAHO, WDC)

Dr. Francisco Alvarado-Ramy, USA (Division of Global Migration and Quarantine, CDC)

B. Laboratory:

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Dr. Pedro Vasconcelos: Brazil (Instituto Evandro Chagas, WHOCC)

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Dr. Robert Lanciotti, USA (Arboviral Diseases Branch, DVBD, CDC)

Dr. César Cabezas, Peru (Instituto Nacional de Salud)

Dr. Erick Halsey, Peru (NMRC, Virology Department)

Dr. Otavio Oliva, USA (PAHO, WDC)

C. Entomology:

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Dr. Juan Arredondo, Mexico (Secretariat of Health)

Dr. Roger Nasci, USA (Arboviral Diseases Branch, DVBD, CDC)

Dr. Harry Savage, USA (Arboviral Diseases Branch, DVBD, CDC)

Dr. Roberto Barrera, Puerto Rico (Dengue Branch, CDC, WHOCC)

Dr. Christian Frederickson, Trinidad and Tobago (PAHO-CAREC)

Dr. José Luis San Martín, Panama (PAHO, Panamá)

D. Clinical Management:

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Dr. Rivaldo Venancio Da Cunha, Brazil (Secretariat of Health)

Dr. Fabrice Simon, France (Department of Infectious Diseases and Tropical Medicine. Laveran Military Hospital)

Dr. Iris Villalobos Chacon, Venezuela (Secretaria de Salud)

Dr. Roser Gonzalez, USA (PAHO, WDC)

E. Social Communication:

Lic. Xinia Bustamante, Costa Rica (PAHO/WHO, Costa Rica)

Dr. Carmen Pérez: Puerto Rico (Dengue Branch, CDC, WHOCC)

Mr. Lee Smith, USA (Division of Global Migration and Quarantine, CDC)

Dr. Marco Fidel Suárez, Bolivia (PAHO/WHO Bolivia)

Dr. Emily Zielinski-Gutierrez, USA (DVBD, CDC) (Final revision)

REFERENCES

1. Lumsden WHR. An epidemic of virus disease in Southern Province, Tanganyika Territory, in 1952-53: II. General description and epidemiology. *Trans R Soc Trop Med Hyg.* 1955;49(1):33-57.
2. Shah KV, Gibbs CJ, Jr., Banerjee G. Virological investigation of the epidemic of haemorrhagic fever in Calcutta: isolation of three strains of Chikungunya virus. *Indian J Med Res.* Jul 1964;52:676-683.
3. Padbidri VS, Gnaneswar TT. Epidemiological investigations of chikungunya epidemic at Barsi, Maharashtra state, India. *J Hyg Epidemiol Microbiol Immunol.* 1979;23(4):445-451.
4. Angelini P, Macini P, Finarelli AC, et al. Chikungunya epidemic outbreak in Emilia-Romagna (Italy) during summer 2007. *Parassitologia.* Jun 2008;50(1-2):97-98.
5. CDC. Chikungunya fever diagnosed among international travelers--United States, 2005-2006. *MMWR Morb Mortal Wkly Rep.* Sep 29 2006;55(38):1040-1042.
6. Queyriaux B, Simon F, Grandadam M, Michel R, Tolou H, Boutin JP. Clinical burden of chikungunya virus infection. *Lancet Infect Dis.* Jan 2008;8(1):2-3.
7. Moro ML, Gagliotti C, Silvi G, et al. Chikungunya virus in North-Eastern Italy: a seroprevalence survey. *Am J Trop Med Hyg.* Mar 2010;82(3):508-511.
8. Borgherini G, Poubeau P, Staikowsky F, et al. Outbreak of chikungunya on Réunion Island: early clinical and laboratory features in 157 adult patients. *Clin Infect Dis.* Jun 1 2007;44(11):1401-1407.

9. Staikowsky F, Le Roux K, Schuffenecker I, et al. Retrospective survey of Chikungunya disease in Réunion Island hospital staff. *Epidemiol Infect.* Feb 2008;136(2):196-206.

10. Taubitz W, Cramer JP, Kapaun A, et al. Chikungunya fever in travelers: clinical presentation and course. *Clin Infect Dis.* Jul 1 2007;45(1):e1-4.

11. Jupp PG, McIntosh BM. Chikungunya virus disease. In: Monath TP, ed. *The Arboviruses: Epidemiology and Ecology.* Vol II. Boca Raton, FL: CDC Press, Inc.; 1988.

12. Staikowsky F, Talarmin F, Grivard P, et al. Prospective study of Chikungunya virus acute infection in the Island of La Réunion during the 2005-2006 outbreak. *PLoS One.* 2009;4(10):e7603.

13. Simon F, Savini H, Parola P. Chikungunya: a paradigm of emergence and globalization of vector-borne diseases. *Med Clin North Am.* 2008;92(6):1323-1343.

14. Pialoux G, Gauzere BA, Jaureguiberry S, Strobel M. Chikungunya, an epidemic arbovirosis. *Lancet Infect Dis.* 2007;7(5):319-327.

15. Sam IC, AbuBakar S. Chikungunya virus infection. *Med J Malaysia.* 2006;61(2):264-269.

16. Lakshmi V, Neeraja M, Subbalaxmi MV, et al. Clinical features and molecular diagnosis of Chikungunya fever from South India. *Clin Infect Dis.* 2008;46(9):1436-1442.

17. Rezza G, Nicoletti L, Angelini R, et al. Infection with chikungunya virus in Italy: an outbreak in a temperate region. *Lancet.* 2007;370(9602):1840-1846.

18. Mavalankar D, Shastri P, Bandyopadhyay T, Parmar J, Ramani KV. Increased mortality rate associated with chikungunya epidemic, Ahmedabad, India. *Emerg Infect Dis.* 2008;14(3):412-415.

19. Beesoon S, Funkhouser E, Kotea N, Spielman A, Robich RM. Chikungunya fever, Mauritius, 2006. *Emerg Infect Dis.* 2008;14(2):337-338.

20. Rajapakse S, Rodrigo C, Rajapakse A. Atypical manifestations of chikungunya infection. *Trans R Soc Trop Med Hyg.* 2010;104(2):89-96.

21. Lewthwaite P, Vasanthapuram R, Osborne JC, et al. Chikungunya virus and central nervous system infections in children, India. *Emerg Infect Dis.* 2009;15(2):329-331.

22. Robin S, Ramful D, Zettor J, et al. Severe bullous skin lesions associated with chikungunya virus infection in small infants. *Eur J Pediatr.* 2010;169(1):67-72.

23. Economopoulou A, Dominguez M, Helynck B, et al. Atypical chikungunya virus infections: clinical manifestations, mortality aud risk factors for severe disease during the 2005-2006 outbreak on Réunion. *Epidemiol Infect.* 2009;137(4):534-541.

24. Lemant J, Boisson V, Winer A, et al. Serious acute chikungunya virus infection requiring intensive care during the Réunion Island outbreak in 2005-2006. *Crit Care Med.* 2008;36(9):2536-2541.

25. Gerardin P, Barau G, Michault A, et al. Multidisciplinary prospective study of mother-to-child chikungunya virus infections on the island of La Réunion. *PLoS Med.* 2008;5(3):e60.

26. Touret Y, Randrianaivo H, Michault A, et al. [Early maternal-fetal transmission of the chikungunya virus]. *Presse Med.* 2006;35(11 Pt 1):1656-1658.

27. Renault P, Solet JL, Sissoko D, et al. A major epidemic of chikungunya virus infection on Réunion Island, France, 2005-2006. *Am J Trop Med Hyg.* 2007;77(4):727-731.

28. Fritel X, Rollot O, Gerardin P, et al. Chikungunya virus infecrion during pregnancy, Réunion, France, 2006. *Emerg Infect Dis.* 2010;16(3):418-425.

29. Robillard PY, Boumahni B, Gerardin P, et al. [Vertical maternal fetal transmission of the chikungunya virus. Ten cases among 84 pregnant women]. *Presse Med.* 2006;35(5 Pt 1):785-788.

30. Ramful D, Carbonnier M, Pasquet M, et al. Mother-to-child transmission of chikungunya virus infection. *Pediatr Infect Dis J.* 2007;26(9):811-815.

31. Das T, Jaffar-Bandjee MC, Hoarau JJ, et al. Chikungunya fever: CNS infection and pathologies of a re-emerging arbovirus. *Prog Neurobiol.* 2010;91(2):121-129.

32. Nimmannitya S, Halstead SB, Cohen SN, Margiotta MR. Dengue and chikungunya virus infection in man in Thailand, 1962-1964. I. Observations on hospitalized patients with hemorrhagic fever. *Am J Trop Med Hyg.* 1969;18(6):954-971.

33. Hochedez P, Canestri A, Guihot A, Brichler S, Bricaire F, Caumes E. Management of travelers with fever and exanthema, notably dengue and chikungunya infections. *Am J Trop Med Hyg.* 2008;78(5):710-713.

34. Staples JE, Breiman RF, Powers AM. Chikungunya fever: an epidemiological review of a re-emerging infectious disease. *Clin Infect Dis.* 2009;49(6):942-948.

35. Brighton SW, Prozesky OW, de la Harpe AL. Chikungunya virus infection. A retrospective study of 107 cases. *S Afr Med J.* 1983;63(9):313-315.

36. Fourie ED, Morrison JG. Rheumatoid arthritic syndrome after chikungunya fever. *S Afr Med J.* 1979;56(4):130-132.

37. Manimunda SP, Vijayachari P, Uppoor R, et al. Clinical progression of chikungunya fever during acute and chronic arthritic stages and the changes in joint morphology as revealed by imaging. *Trans R Soc Trop Med Hyg.* 2010;104(6):392-399.

38. Sissoko D, Malvy D, Ezzedine K, et al. Post-epidemic chikungunya disease on Réunion Island: course of rheumatic manifestations and associated factors over a 15-month period. *PLoS Negl Trop Dis.* 2009;3(3):e389.

39. Soumahoro MK, Gerardin P, Boelle PY, et al. Impact of chikungunya virus infection on health status and quality of life: a retrospective cohort study. *PLoS One.* 2009;4(11):e7800.

40. Bouquillard E, Combe B. Rheumatoid arthritis after Chikungunya fever: a prospective follow-up study of 21 cases. *Ann Rheum Dis.* 2009;68(9):1505-1506.

41. Hoarau JJ, Jaffar Bandjee MC, Trotot PK, et al. Persistent chronic inflammation and infection by Chikungunya arthritogenic alphavirus in spite of a robust host immune response. *J Immunol.* 2010;184(10):5914-5927.

42. Jayakeerthi RS, Potula RV, Srinivasan S, Badrinath S. Shell vial culture assay for the rapid diagnosis of Japanese encephalitis, West Nile and Dengue-2 viral encephalitis. *Virol J.* 2006;3:2.

43. Lanciotti RS, Kosoy OL, Laven JJ, et al. Chikungunya virus in US travelers returning from India, 2006. *Emerg Infect Dis.* 2007;13(5):764-767.

44. Martin DA, Muth DA, Brown T, Johnson AJ, Karabatsos N, Roehrig JT. Standardization of immunoglobulin M capture enzyme-linked immunosorbent assays for routine diagnosis of arboviral infections. *J Clin Microbiol.* 2000;38(5):1823-1826.

45. Brighton SW. Chloroquine phosphate treatment of chronic chikungunya arthritis. An open pilot study. *S Afr Med J.* 1984;66(6):217-218.

46. De Lamballerie X, Boisson V, Reynier JC, et al. On chikungunya acute infection and chloroquine treatment. *Vector Borne Zoonotic Dis.* 2008;8(6):837-839.

47. Cordel H, Quatresous I, Paquet C, Couturier E. Imported cases of chikungunya in metropolitan France, April 2005 - February 2006. *Euro Surveill.* 2006;11(4):E060420 060423.

48. U.S. DHHS. *Biosafety in microbiological and biomedical laboratories.* 4th ed. Washington, DC: US Government Printing Office; 1999.

49. PAHO. PAHO Strategic and operational plan for responding to pandemic influenza. 2005. http://www.paho.org/English/AD/PAHO_Plan_PandemicInfluenza_Eng.pdf. Accessed 11 August 2010.

50. U.S. DHHS. HHS Pandemic Influenza Plan; 2005. www.hhs.gov/pandemicflu/plan/pdf/HHSPandemicInfluenzaPlan.pdf. Accessed 2 June 2010.

51. WHO. Guidelines on Clinical Management of Chikungunya Fever; 2008. http://www.searo.who.int/LinkFiles/Publication_guidelines_on_cli_mgmt_chikungunya_fvr-(cd-180).pdf. Accessed 2 June 2010.

52. Petersen LR, Stramer SL, Powers AM. Chikungunya virus: possible impact on transfusion medicine. *Transfus Med Rev.* 2010;24(1):15-21.

53. Liumbruno GM, Calteri D, Petropulacos K, et al. The chikungunya epidemic in Italy and its repercussion on the blood system. *Blood Transfus.* 2008;6(4):199-210.

54. Sissoko D, Ezzedine K, Moendandze A, Giry C, Renault P, Malvy D. Field evaluation of clinical features during chikungunya outbreak in Mayotte, 2005-2006. *Trop Med Int Health.* 2010;15(5):600-607.

55. WHO. International Health Regulations (2005); 2008. http://whqlibdoc.who.int/publications/2008/9789241580410_eng.pdf. Accessed 2 June 2010.

56. WHO. Prevention and Control of Chikungunya in South-East Asia. *Report of the Expert Group Meeting.* Aurangabad, India: Regional Office World Health Organization; 2008. http://www.searo.who.int/LinkFiles/Publicarion_CD-176.pdf. Accessed 2 June 2010.

57. Dubrulle M, Mousson L, Moutailler S, Vazeille M, Failloux AB. Chikungunya virus and *Aedes* mosquitoes: saliva is infectious as soon as two days after oral infection. *PLoS One.* 2009;4(6):e5895.

58. Schuffenecker I, Iteman I, Michault A, et al. Genome microevolution of chikungunya viruses causing the Indian Ocean outbreak. *PLoS Med.* 2006;3(7):e263.

59. de Lamballerie X, Leroy E, Charrel RN, Ttsetsarkin K, Higgs S, Gould EA. Chikungunya virus adapts to tiger mosquito via evolutionary convergence: a sign of things to come? *Virol J.* 2008;5:33.

60. Arias JR. Dengue: How are we doing? *Celebrating 100 Years of PAHO.* Washington, D.C.: PAHO; 2002.

61. Benedict MQ, Levine RS, Hawley WA, Lounibos LP. Spread of the tiger: global risk of invasion by the mosquito *Aedes albopictus. Vector Borne Zoonotic Dis.* 2007;7(1):76-85.

62. Niebylski ML, Savage HM, Nasci RS, Craig GB Jr. Blood hosts of *Aedes albopictus* in the United States. *J Am Mosq Control Assoc.* 1994;10(3):447-450.

63. Hawley WA. The biology of *Aedes albopictus*. *J Am Mosq Control Assoc Suppl.* 1988;1:1-39.

64. Savage HM, Smith GC. Identification of damaged adult female specimens of *Aedes albopictus* and *Aedes aegypti* in the New World. *J Am Mosq Control Assoc.* 1994;10(3):440-442.

65. Darsie RFJ, Ward RA. *Identification and geographical distribution of the mosquitoes of North America, New Mexico.* Gainesville, FL: University Press of Florida; 2005.

66. WHO. Dengue Guidelines for Diagnosis, Treatment, Prevention and Control; 2009. http://whqlibdoc.who.int/publications/2009/9789241547871_eng.pdf. Accessed 2 June 2010.

67. Parks W, Lloyd L. Planning social mobilization and communications for dengue fever prevenrion and control: a step-by-step guide; 2004. http://apps.who.int/tdr/publications/training-guideline-publications/planning-social-mobilization-dengue-fever/pdf/planning_dengue.pdf. Accessed 2 June 2010.

68. WHO. Global strategic framework for integrated vector management. 2004. http://whqlibdoc.who.int/hq/2004/WHO_CDS_CPE_PVC_2004_10.pdf. Accessed 11 August 2010.

69. Barrera R, Delgado N, Jimenez M, Villalobos I, Romero I. [Stratification of a hyperendemic city in hemorrhagic dengue]. *Rev Panam Salud Publica.* 2000;8(4):225-233.

70. Williams CR, Long SA, Webb CE, et al. *Aedes aegypti* population sampling using BG-Sentinel traps in north Queensland Australia: statistical considerations for trap deployment and sampling strategy. *J Med Entomol.* 2007;44(2):345-350.

71. Barrera R. Simplified pupal surveys of *Aedes aegypti* (L.) for entomologic surveillance and dengue control. *Am J Trop Med Hyg.* 2009;81(1):100-107.

72. Lenhart A, Orelus N, Maskill R, Alexander N, Streit T, McCall PJ. Insecticide-treated bednets to control dengue vectors: preliminary evidence from a controlled trial in Haiti. *Trop Med Int Health.* 2008;13(1):56-67.

73. Kroeger A, Lenhart A, Ochoa M, et al. Effective control of dengue vectors with curtains and water container covers treated with insecticide in Mexico and Venezuela: cluster randomised trials. *BMJ*. 2006;332(7552):1247-1252.

74. Morrison AC, Zielinski-Gutierrez E, Scott TW, Rosenberg R. Defining challenges and proposing solutions for control of the virus vector *Aedes aegypti*. *PLoS Med*. 2008;5(3):e68.

75. Erlanger TE, Keiser J, Utzinger J. Effect of dengue vector control interventions on entomological parameters in developing countries: a systematic review and meta-analysis. *Med Vet Entomol*. 2008;22(3):203-221.

76. Esu E, Lenhart A, Smith L, Horstick O. Effectiveness of peridomestic space spraying with insecticide on dengue transmission; systematic review. *Trop Med Int Health*. 2010;15(5):619-631.

77. WHO. Pesticides and their application: For the control of vectors and pests of public health importance; 2006. http://whqlibdoc.who.int/hq/2006/WHO_CDS_NTD_WHOPES_GCDPP_2006.1_eng.pdf. Accessed 2 June 2010.

78. Nauen R. Insecticide resistance in disease vectors of public health importance. *Pest Manag Sci*. 2007;63(7):628-633.

79. Montella IR, Martins AJ, Viana-Medeiros PF, Lima JB, Braga IA, Valle D. Insecticide resistance mechanisms of Brazilian *Aedes aegypti* populations from 2001 to 2004. *Am J Trop Med Hyg*. 2007;77(3):467-477.

80. Rodriguez MM, Bisset JA, Fernandez D. Levels of insecticide resistance and resistance mechanisms in *Aedes aegypti* from some Latin American countries. *J Am Mosq Control Assoc*. 2007;23(4):420-429.

81. Hemingway J. *Techniques to detect insecticide resistance mechanisms (Field and Laboratory Manual)*. Geneva, Switzerland: WHO; 1998.

82. Brogdon WG. Biochemical resistance detection: an alternative to bioassay. *Parasitol Today*. 1989;5(2):56-60.

83. Brogdon WG, McAllister JC. Insecticide resistance and vector control. *Emerg Infect Dis*. 1998;4(4):605-613.

84. Hills SL, Piispanen JP, Humphreys JL, Foley PN. A focal, rapidly-controlled outbreak of dengue fever in two suburbs in Townsville, north Queensland, 2001. *Commun Dis Intell.* 2002;26(4):596-600.

85. Morrison AC, Getis A, Santiago M, Rigau-Perez JG, Reiter P. Exploratory space-time analysis of reported dengue cases during an outbreak in Florida, Puerto Rico, 1991-1992. *Am J Trop Med Hyg.* 1998;58(3):287-298.

86. WHO. *Safe use of pesticides. Fourteenth report of WHO Expert Committee on Vector Biology and Control.* Geneva, Switzerland 1991. 813.

87. Barrera R, Amador M, Diaz A, Smith J, Munoz-Jordan JL, Rosario Y. Unusual productivity of *Aedes aegypti* in septic tanks and its implications for dengue control. *Med Vet Entomol.* 2008;22(1):62-69.

88. WHO. Guidelines for drinking-water quality, 3rd ed.; 2006. http://www.who.int/water_sanitation_health/dwq/gdwq3rev/en/index.html. Accessed 2 June 2010.

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Eastern Equine Encephalitis Virus Surveillance and Control Guidelines

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Appendix 1. EEE Virus Testing for for Mosquito Pools (Real-Time RT-PCR)

About These Guidelines

Comprehensive CDC guidelines for arbovirus surveillance programs in the United States were published in 1993 (CDC 1993). These guidelines detailed best practices for surveillance and control of eastern equine encephalitis (EEE), La Crosse encephalitis (LAC), St. Louis encephalitis (SLE), and western equine encephalitis (WEE). In the several decades since the guidelines were published, EEE has emerged as a vector-borne disease of increasing public health concern due to multiple outbreaks of neuroinvasive disease and the potential for further expansion of EEE into new U.S. regions. Additionally, knowledge about EEE epidemiology and transmission ecology has greatly expanded. The objective of this guidance is to consolidate new knowledge and describe how this can be used to better assess EEE virus activity and mitigate its public health impact. These guidelines are meant for state and local public health officials and mosquito control personnel to aid them in the surveillance and control of EEE.

References

CDC. Guidelines for arbovirus surveillance programs in the United States. [PDF – 85 pages] Fort Collins. 1993

Last Reviewed: December 5, 2022

Appendix 1. EEE Virus Testing for for Mosquito Pools (Real-Time RT-PCR)

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Before conducting any EEE virus testing, note:

EEE virus is an HHS Select Agent, and therefore, subject to strict regulations regarding its possession and use. Those intending to conduct EEE virus testing must be familiar with the complete information and specific guidance found at the Federal Select Agent Program website ***before*** conducting EEE virus testing.

Briefly, samples determined to be positive for EEE virus must be documented and reported to the Federal Select Agent Program via Form 4 (https://www.selectagents.gov/form4.html) within 7 calendar days of identification, and, if not diagnosed at a registered entity, they must then be transferred to a registered Select Agent facility or destroyed.

Testing Algorithm. All samples are screened for virus using either or both sets of the primers/probes listed below. A positive result in any of the negative controls invalidates the entire run. Failure of the positive control to generate a positive result also invalidates the entire run. A sample that is positive with one primer set and negative with the second set is classified as equivocal.

Note: At the CDC, Division of Vector-borne Diseases, Arboviral Disease Branch, the kits and protocols used by the Entomology and Ecology team are described below; however, there are several other options for RNA extraction and real-time RT-PCR on the market.

Results Interpretation

We use the following algorithm to evaluate the results.

Positive: Ct value ≤ 37

Negative: Ct value > 37

PCR PLATE SET-UP:

1. Prepare primers and probes according to the following concentrations:
   - Primers: 100 µM in nuclease-free water
   - Probes: 25 µM in TE buffer
2. Real-time RT-PCR master mix should be prepared in a "clean room" physically separated from all other laboratory activities with dedicated reagents and equipment (i.e., pipettes). Combine the reagents listed below in an RNase free centrifuge tube on ice. Using Qiagen's Quantitect Probe RT-PCR kit (#204443), prepare master mix as follows:Per reaction:

   - 0 µl master-mix
   - 2 µl water* (nuclease-free)

- 5 µl 100µM forward primer
- 5 µl 100µM reverse primer
- 3 µl 25µM probe
- 5 µl RT enzyme
  Add about 5-10 reactions to your total number of samples (and account for "No template controls" (NTCs), positive controls, and negative extraction controls) and multiply number by volumes above. Example: You have 20 samples (12 unknown samples, 2 positive controls, 2 negative controls, and 4 NTCs). Make a master mix for 25 to 30 samples.
- NTC = mix ONLY with no sample, to test mix components (*PCR control*)
- Negative control = extracted water (*extraction control*)

3. Pipette **45 µl of master mix*** into either 0.2 ml optical (specifically for real-time assays; emission fluorescence is read through the cap) PCR tubes or a 96-well optical PCR plate. Use a reservoir and a multichannel pipette for many wells.
4. Pipette **5 µl of RNA*** into each well. Refer to a template to ensure that the proper sample is added to the corresponding well. Do not add anything to NTC samples (master mix only).
   - See RNA extraction tips below.

**The volume of RNA added per reaction is typically 5 µl but can be increased (up to 25 µl) with the appropriate adjustment of the water in the master mix. For example, if you want to test 10 µl RNA, reduce the water per reaction to 13.2 µl, and add 40 µl master mix and 10 µl RNA to each well.*

Cycling conditions (QIAGEN conditions for Real Time RT-PCR):

1 cycle each:

50°C for 30 min

95°C for 15 min

45 cycles:

95°C for 15 sec

60°C for 1 min (data collection step)

EEEV primers and probes. There are one published and one unpublished primer/probe sets available for the detection of EEEV RNA.

Published: Lambert et al. 2003.

EEEV 9391 F ACACCGCACCCTGATTTTACA

EEEV 9459 R CTTCCAAGTGACCTGGTCGTC

EEEV 9414-probe TGCACCCGGACCATCCGACCT

(unpublished)

EEEV 1898 F ACCTTGCTGACGACCAGGTC

EEEV 1968 R GTTGTTGGTCGCTCAATCCA

EEEV 1919-probe CTTGGAAGTGATGCAAATCCACTCGACA

Rna Extraction Tips

NOTES: Avoid contamination while working with RNA

- Maintain physically separated work areas; one dedicated to **pre-amplification RNA work** (RNA extraction) and the other for **master mix** production.
- Utilize dedicated/separate equipment within pre and post amplification areas; especially pipettes and centrifuges.
- Always wear gloves; even when handling unopened tubes.
- Open and close tubes quickly and avoid touching any inside portion.
- Use RNase free plastic disposable tubes and pipet tips.
- Use aerosol block pipet tips.
- Use RNase free water.
- Prepare all reagents on ice.

1. Solid phase samples (mosquitoes or tissues) are first homogenized in an isotonic buffer to produce a liquid homogenate. Mosquito specimens are homogenized using copper clad steel bead (BB) grinding technique using a vortexer or mixer mill (i.e., Qiagen Tissuelyser). Homogenates are clarified by centrifugation in a microcentrifuge (i.e., Eppendorf) at maximum speed for 5 minutes to pellet any particulate material.
2. Extract RNA from the clarified supernatant using the QiaAmp viral RNA kit (QIAGEN part #52904) or another comparable kit specifically designed to purify RNA. Follow the manufacturer's protocol exactly with the following modification for mosquito specimens: include 1 additional wash/centrifugation step with AW1, if using the Qiagen kit. Extract at least two negative controls and two positive controls along with the test specimens. The positive controls should differ in the amount of target RNA present (i.e., a pre-determined high positive and a low positive). Note: The volume of sample extracted can be greater or less than the standard volume stated in the QIAGEN protocol (140 µl) with the appropriate adjustments to all other volumes in the protocol. CDC typically extracts 100 µl.

References

Lambert AJ, et al. Detection of North American eastern and western equine encephalitis viruses by nucleic acid amplification assays. *J. Clin. Microbiol* 2003; 41:379-385.

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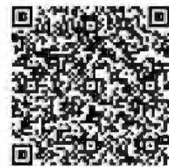

Mosquitoes

ArboNET

ArboNET, the national arboviral surveillance system, was developed by CDC and state health departments in 2000 in response to the emergence of WNV in 1999. Since its development, ArboNET has expanded to include many other arboviruses of public health importance. ArboNET is an electronic surveillance system administered by CDC's Division of Vector-Borne Diseases (DVBD). Human arboviral disease data are reported from all states, territories, and associated states. In addition to human disease cases, ArboNET maintains data on arboviral infections among human viremic blood donors, non-human mammals, sentinel animals, dead birds, and mosquitoes.

Data Collected. Variables collected for human disease cases include patient age, sex, race, and county and state of residence; date of illness onset; case status (i.e., confirmed, probable, suspected, or not a case); clinical syndrome (e.g., encephalitis, meningitis, or uncomplicated fever); whether illness resulted in hospitalization; and whether the illness was fatal. Cases reported as encephalitis (including meningoencephalitis), meningitis, or acute flaccid paralysis are collectively referred to as neuroinvasive disease; others are considered non-neuroinvasive disease. Acute flaccid paralysis can occur with or without encephalitis or meningitis. Information regarding potential non-mosquito-borne transmission (e.g., blood transfusion or organ transplant recipient, breast-fed infant, or laboratory worker) and recent donation of blood or solid organs should be reported if applicable. Clinical symptoms and diagnostic testing data can also be reported.

Blood donors identified as presumptively viremic by nucleic acid amplification test (NAAT) screening of the donation by a blood collection agency are also reported to ArboNET. Case definitions have been developed for the purposes of national surveillance. The date of blood donation is reported in addition to the variables routinely reported for disease cases.

Arboviral disease in non-human mammals (primarily horses) and infections in trapped mosquitoes, dead birds, and sentinel animals (primarily chickens) are also reported to ArboNET. Variables collected for non-human infections include species, state and county, and date of specimen collection or symptom onset. Until 2023, the total number of mosquitoes or birds tested weekly also could be reported by county and species.

Detailed descriptions of all variables collected by ArboNET and instructions for reporting are included in the ArboNET User Guide, which can be requested from DVBD by phone (970-261-6400) or email (dvbid2@cdc.gov).

Data Transmission. Jurisdictions can transmit data to ArboNET using one or more of four methods supported by DVBD: 1) jurisdictions that have a commercially- or state-developed electronic surveillance system can upload records from their system using an Extensible Markup Language (XML) message; 2) jurisdictions can upload records from a Microsoft Access database provided by CDC DVBD using an XML message; 3) jurisdictions may enter records manually using a CDC website (https://csams.cdc.gov/arbonet); or 4) jurisdictions can report cases using an HL-7 message via the CDC National Electronic Disease Surveillance System (NEDSS), and DVBD will download records directly from NEDSS to ArboNET. ArboNET data are maintained in a Microsoft Structured Query Language (SQL) Server database inside CDC's firewall (note this is likely to change soon and potentially during the 2023 season). Users can access data via a password-protected website but are limited to viewing data only from their own jurisdiction. The ArboNET website and database are maintained by CDC information technology staff and are backed up nightly.

Dissemination of ArboNET Data. CDC epidemiologists periodically review and analyze ArboNET surveillance data and disseminate results to stakeholders via direct communication, briefs in *Morbidity and Mortality Weekly Reports* and Epi-X, comprehensive annual summary reports, and DVBD's website. CDC also produces maps of domestic and exotic arboviral

activity, which are then posted on a website (https://csams.cdc.gov/arbonet/maps/adb_diseases_map/index.html). Surveillance reports are typically updated biweekly during the transmission season and monthly during the off-season. A final report is usually released in the spring of the following year. CDC provides limited-use ArboNET data sets to the general public by formal request. Data release guidelines have been updated to be consistent with those developed by CDC and the Council of State and Territorial Epidemiologists.

Limitations of ArboNET Data. Human surveillance for arboviral disease is largely passive and relies on the receipt of information from physicians, laboratories, and other reporting sources by state health departments. For viruses that can cause neuroinvasive disease, neuroinvasive disease cases are likely to be consistently reported because of the substantial morbidity associated with this clinical syndrome. In comparison, non-neuroinvasive disease cases are inconsistently reported because of a less severe spectrum of illness, geographic differences in disease awareness and healthcare-seeking behavior, and variable capacity for laboratory testing. Surveillance data for fever cases associated with neuroinvasive arboviruses should be interpreted with caution and generally should not be used to make comparisons between geographic areas or over time. Accordingly, ratios of reported neuroinvasive disease cases to non-neuroinvasive disease cases should not be interpreted as a measure of virulence in an area.

ArboNET does not routinely collect information regarding clinical signs and symptoms or diagnostic laboratory test results. Therefore, misclassification of the various syndromes caused by arboviruses cannot be detected. In addition, ArboNET does not routinely collect information regarding the specific laboratory methods used to confirm each case. Although serologic assays are relatively specific, false-positive results and cross-reactions occur between related viruses (e.g., flavivirus, such as West Nile, SLE, and dengue viruses, or California serogroup viruses, such as La Crosse and Jamestown Canyon viruses). Positive IgM antibody results should be confirmed by additional tests, especially plaque-reduction neutralization test (PRNT). However, such confirmatory testing often is not performed. While the electronic mechanisms for data transmission allow for rapid case reporting, the inclusion of both clinical and laboratory criteria in the surveillance case definition creates delays between the occurrence of cases and their reporting. Provisional data are disseminated to allow for monitoring of regional and national epidemiology during the arboviral transmission season. However, these reports generally lag several weeks behind the occurrence of the cases comprising them, and the data may change substantially before they are finalized. For this reason, provisional data from the current transmission season should not be combined with or compared to provisional or final data from previous years.

The collection and reporting of non-human surveillance data are highly variable among states (and even between regions within states) and changes from year to year. Because of this variability, non-human surveillance data should not be used to compare arboviral activity between geographic areas or over time.

For more information about ArboNET, please contact the Division of Vector-Borne Diseases by phone: 970-261-6400 or email: dvbid2@cdc.gov.

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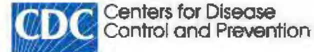

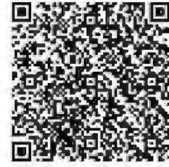

Ecology and Epidemiology

Ecology and Epidemiology

Eastern equine encephalitis (EEE) virus is an alphavirus endemic to the United States. EEE virus is maintained in the environment mainly by cycling between birds and mosquitoes inhabiting freshwater hardwood swamps (Morris 1988).

Transmission risk is highest in lowland regions where its principal enzootic vector, the ornithophilic mosquito *Culiseta melanura* readily occurs. Most cases occur in eastern or Gulf Coast states. As with other zoonotic arboviruses, EEE virus persists in a complex ecological web of host species, alternating between mosquito vectors and vertebrate amplification hosts. Habitats that pose a threat to humans and other vertebrates are those that support mosquito species that can serve as vectors and vertebrate hosts that develop viremia of sufficient magnitude to infect mosquitoes (amplification hosts). Reptiles and amphibians have also been implicated in enzootic transmission, particularly in Southern states, perhaps serving as an over-wintering refuge for EEE virus (Graham et al. 2012).

Freshwater swamp and bog habitats are a source ecosystem for EEE virus even in periods of low-level transmission (Miley et al. 2021, Skaff et al. 2021). These freshwater habitats provide pools of water for *Cs. melanura* larval growth and development and plant nectar for adult mosquito survival. In the Northeast region, EEE virus infections in mosquitoes are correlated with proximity to forested wetland habitat dominated by red maple (*Acer rubrum*) and Atlantic white cedar (*Chamaecyparis thyoides*). The dominant trees shift to bald cypress (*Taxodium distichum*) and water tupelo (*Nyssa aquatica*) in the southeast bottomlands. In the Great Lakes region, *Cs. melanura* favor sphagnum bogs for oviposition and larval development. Primary buttressed trees in these habitats include black spruce, red spruce, yellow birch, and tupelo.

In the late summer, bird populations disperse after breeding, causing declines in easily available avian blood meals. Swamp-inhabiting mosquitoes may then seek blood outside of the swamp habitats, increasing risk that the virus will spill over to infect other vertebrates and secondary vectors. Surveillance for EEE virus-infected hosts routinely detects vertebrate and vector infections within the swamp habitat, and during epizootic and epidemic transmission outside their primary habitat. Some secondary vectors act as bridge vectors, feeding on birds and mammals and transmitting virus from enzootic to epizootic and epidemic hosts. Once spillover occurs, surveillance can detect EEE virus infections in other potential vector mosquito species.

Vectors suspected of transmitting EEE virus to horses and humans vary geographically. *Culiseta melanura* can act as both an enzootic and epidemic vector (Armstrong and Andreadis 2010). Primary bridge vectors include *Coquillettidia perturbans, Aedes canadensis, Ae. sollicitans,* and *Culex salinarius* in the Northeast and Mid-Atlantic regions (Armstrong and Andreadis 2010; Armstrong and Andreadis 2022; Crans 1977), *Cq. perturbans, Ae. canadensis, Cx. salinarius,* and *Cx. erraticus* in the Southeast (Burkett-Cadena et al. 2022), and *Cq. perturbans* in the Midwest (Nasci et al. 1993). Bridge vectors are competent to transmit virus and infected mosquitoes of these species are frequently detected during epizootic and epidemic periods. Horses and humans are considered dead-end hosts because they do not develop high enough levels of EEE virus in their blood to infect feeding mosquitoes.

The first human EEE disease cases were recognized during a 1938 outbreak in southeastern Massachusetts (Feemster 1938). Subsequent outbreaks were then reported in New Jersey during the 1950s (Goldfield and Sussman 1968). Since then, EEE cases have occurred sporadically and in small clusters, most around freshwater hardwood swamps in the Atlantic and Gulf Coast states and the Great Lakes region (Goldfield and Sussman 1968; Przelomski 1988; CDC 2006). Human infections typically occur during summer months, with >80% of reported human cases having an illness onset

during July through September (Lindsey et al. 2018). During 2003–2018, an average of eight EEE disease cases were reported annually in the United States (range = 4–21 cases/year) (Lindsey 2018; CDC 2021). However, in 2019, 38 cases were reported nationally (Vahey et al. 2021). The reasons for this increase are unknown but are likely related to several factors, including weather, abundance of birds and mosquitoes that can transmit the virus, human behavior, and clinical awareness and diagnostic testing practices.

References

Armstrong PM and Andreadis TG. Eastern equine encephalitis virus in mosquitoes and their role as bridge vectors. *Emerg Infect Dis* 2010;16(12):1869–74.

Armstrong PM and Andreadis TG. Ecology and epidemiology of eastern equine encephalitis virus in the Northeastern United States: An historical perspective. *J Med Entomol* 2022;59(1):1–13.

Burkett-Cadena ND, et al. Ecology of eastern equine encephalitis virus in the Southeastern United States: Incriminating vector and host species responsible for virus amplification, persistence, and dispersal. *J Med Entomol* 2022;59(1):41–48.

CDC. Eastern equine encephalitis–New Hampshire and Massachusetts, August–September 2005. *MMWR* 2006;55(25):697–700.

CDC. Eastern equine encephalitis virus: Statistics & Maps. Fort Collins; 2021. https://www.cdc.gov/easternequineencephalitis/statistics-maps/index.html.

Crans WJ. The status of *Aedes sollicitans* as an epidemic vector of eastern equine encephalitis in New Jersey. *Mosquito News*. 1977;37(1):85–9.

Feemster RF. Outbreak of encephalitis in man due to the eastern virus of equine encephalomyelitis. *Am J Public Health Nations Health* 1938;28:1403–1410.

Goldfield M and Sussman O. The 1959 outbreak of eastern encephalitis in New Jersey. I. Introduction and description of outbreak. *Am J Epidemiol* 1968;87:1–10.

Graham S, et al. Serosurveillance of eastern equine encephalitis virus in amphibians and reptiles from Alabama, USA. *Am J Trop Med Hyg* 2012;86(3):540.

Lindsey NP, et al. Eastern equine encephalitis in the United States, 2003–2016. *Am J Trop Med Hyg* 2018;98:1472–1477.

Miley KM, et al. Field analysis of biological factors associated with sites at high and low to moderate risk for eastern equine encephalitis virus winter activity in Florida. *J Med Entomol* 2021;8:2385–2397.

Morris CD. Eastern equine encephalitis. Monath TP, ed. The arboviruses: *Epidemiology and ecology*, Vol. 3. Boca Raton (FL): CRC Press; 1988. p. 1–20.

Nasci RS, et al. Eastern equine encephalitis virus in Ohio during 1991. *J Med Entomol* 1993;30(1):217–22.

Przelomski MM, et al. Eastern equine encephalitis in Massachusetts: A report of 16 cases, 1970–1984. *Neurology* 1988;38:736–739.

Skaff NK, et al. Wetland characteristics linked to broad-scale patterns in *Culiseta melanura* abundance and eastern equine encephalitis virus infection. *Parasit Vectors* 2021;10:501.

Vahey GM, et al. West Nile virus and other domestic nationally notifiable arboviral diseases–United States, 2019. *MMWR* 2021;70:1069–1074.

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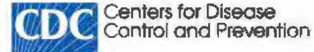

Mosquitoes

Environmental Surveillance

Vector-based Surveillance

Vector surveillance is an integral component of an Integrated Vector Management (IVM) program and is the primary tool for quantifying virus transmission and human risk. The principal functions of a mosquito-based surveillance program are to:

- Collect data on mosquito population abundance and virus infection rates in those populations
- Provide indicators of the threat of human infection and identify geographic areas of high risk
- Support decisions regarding the need for and timing of intervention activities (e.g., enhanced vector surveillance and control, use of new technologies and public education programs)
- Monitor the effectiveness of vector control methods, including susceptibility of target mosquitoes to control methods used

Mosquito-based arboviral monitoring complements disease surveillance programs by contributing timely results and data for action. Programs maintaining in-house laboratories may be able to process mosquito samples daily, giving results within a few days. Data on vector species community composition, relative abundance, and infection rates allow programs to rapidly compute infection indices, assess risk, and respond. Maintaining mosquito surveillance over the long-term provides a baseline of historical data to evaluate risk and guide mosquito control operations. However, the utility of mosquito-based surveillance depends both on the type and quality of data collected (e.g., number and type of traps, timing and frequency of sampling, testing procedures) and consistent effort across transmission seasons to link surveillance indices with human risk.

There are three main categories of vector surveillance: larval, adult, and transmission activity. Together, this information is used to determine where and when control efforts should be implemented. Larval surveillance involves sampling a wide range of aquatic habitats to identify the sources of vector mosquitoes and evaluating larval control measures applied. For adult mosquitoes, regular (e.g., monthly, weekly) sampling is done at fixed sites throughout the community that are representative of the habitat types present in the area. Adult mosquitoes are collected using a variety of trapping techniques, including traps for host-seeking, resting, or gravid (carrying eggs) mosquitoes seeking a place to lay eggs (oviposition site). Adult surveillance can also be used to evaluate control activities pre- and post-treatment. Transmission activity surveillance provides information on the level of infected mosquitoes in an area.

Specimen Collection and Traps

Mosquito species involved in enzootic or epidemic transmission are readily captured in CDC light traps (with or without CO_2) and New Jersey light traps. For best results, the traps need to be placed in well-protected sites with very limited wind movement. Resting boxes may be used to increase the chances of capturing infected mosquitoes, and the CDC battery powered resting box traps can increase the number of mosquitoes captured, as well as improve consistency and ease of sampling (Panella et al. 2011). The resting populations can also be collected using backpack aspirators (e.g., modified CDC backpack aspirator https://www.johnwhock.com/products/aspirators/modified-cdc-backpack-aspirator/ , or the lightweight battery-powered aspirator [Nasci 1981]) to remove mosquitoes from natural harborages or artificial resting structures (e.g., wooden resting boxes, red boxes, fiber pots, and other similar containers (Holderman et al. 2018)).

Specimen Handling and Processing

Because mosquito-based surveillance relies on identifying virus in the collected mosquitoes through detection of viral proteins, viral RNA, or live virus (see Laboratory Diagnosis and Testing section), specimens should be handled in a way that minimizes exposure to conditions (e.g., heat, successive freeze-thaw cycles) that would degrade the virus. Optimally, a cold chain should be maintained from the time mosquitoes are removed from the traps to the time they are delivered to the processing laboratory. Mosquitoes can be transported from the field in a cooler with cold packs or on dry ice, and then placed on a chill-table, if available, during sorting, identification, and pooling. Usually only female mosquitoes are tested in routine arboviral surveillance programs. If virus screening is not done immediately after mosquito identification and pooling, the pooled samples should be stored frozen (e.g., -70°C) or at temperatures below freezing for short-term storage. Although the lack of a cold chain might impact the ability to culture the virus, it does not appear to reduce the ability to detect viral RNA by reverse transcription-polymerase chain reaction (RT-PCR) (Turell et al. 2002).

Vector-based Surveillance Indicators

Data derived from mosquito surveillance include estimates of mosquito species abundance and infection rate in those mosquito populations. The indices derived from those data vary in information content, ability to be compared over time and space, and association with transmission levels and levels of human risk. Five indicators that have commonly been used include: vector abundance, number of positive pools, percent of pools positive, infection rate, and vector index (Table 1).

Vector abundance provides a measure of the relative number of mosquitoes in an area during a particular sampling period. It is the total number of mosquitoes of a particular species collected, divided by the number of trapping nights during a specified sampling period, and is expressed as the number/trap night. Risk assessments often consider mosquito abundance because high mosquito densities can be associated with arboviral disease outbreaks (Olson et al. 1979; Eldridge 2004). For example, during a WNV outbreak in Maricopa County, AZ in 2010, *Culex quinquefasciatus* densities were higher in outbreak compared to non-outbreak areas (Godsey et al. 2012; Colborn et al. 2013). High *Culiseta melanura* and *Coquillettidia perturbans* abundance has also been associated with elevated eastern equine encephalitis (EEE) virus activity. However, high mosquito abundance can occur in the absence of virus, and outbreaks can occur when abundance is low, but the vector infection rate is high. Vector abundance measures are used for planning IVM and monitoring the outcomes of mosquito control. Number of traps, their distribution, and the timing of sample collection should be sufficient to obtain spatially and temporally representative data.

Number of positive pools is the total of the number of arbovirus positive mosquito pools detected in a given surveillance location and period. These may be a tally of the total positive pools separated by species or for all species tested. This indicator provides evidence of arboviral activity, particularly during field investigations and outbreak response, but is not recommended as a stand-alone indicator. Instead, data can be used to produce more informative indices (i.e., infection rate and vector index).

Percent of pools positive is calculated by the number of positive pools divided by the total number of pools tested, as a percentage. It provides data that can be used to compare activity over time and place. However, the comparative value is limited unless the number of pools tested is large and the number of mosquitoes per pool remains constant. As with the number of positive pools index, these data can be used for calculation of the, often more informative, infection rate and vector index.

The **infection rate** in a sampled vector population estimates the true infection prevalence of infected mosquitoes in the population and is a good indicator of human risk. It provides a useful, quantitative basis for comparison, allowing evaluation of changes in population infection prevalence over time and space. Infection rate indices have been used successfully to link infection rates with human risk (Bell et al. 2005). When computing infection rate indices, variable pool numbers and pool sizes can be used, while retaining comparability, but larger sample sizes improve precision. Two methods are commonly used to calculate infection rate:

- **Minimum infection rate (MIR)** for a given mosquito species is the number of positive pools divided by the total number of mosquitoes tested. Use of the MIR assumes that infection rates are low and that only one mosquito is positive in a positive pool.
- **Maximum likelihood estimate (MLE) corrected for bias** is the preferred method, particularly during outbreaks. MLE-associated estimates are based on binomial probability models for pooled data and do not assume only one positive mosquito per positive pool. Bias-corrected MLEs provide more accurate estimates than the standard MLE (Biggerstaff

2008; Hepworth and Biggerstaff, 2017, 2021) and are more accurate than the MIR (Gu et al. 2008; Biggerstaff 2008). MLE-based estimates are computed from straightforward formulas when there is only one pool size, but computer iterative methods are needed when pool sizes differ. Both an R package and a Microsoft Excel Add-in are available to compute infection rate estimates from pooled data (https://www.cdc.gov/westnile/resourcepages/mosqSurvSoft.html).

While the MLE-based estimates and the MIR are similar when infection rates are low, the assumption underlying the use of the MIR is untenable as the true infection rate increases, the MIR is less accurate than bias-corrected MLEs, and in any case confidence intervals based on the MIR have been shown to be poor (e.g., Biggerstaff 2008).

The **Vector Index (VI)** estimates the relative abundance of infected mosquitoes in an area and incorporates into a single index information on presence, relative abundance, and infection rates of individual species (Gujaral et al. 2007; Bolling et al. 2009; Jones et al. 2011). The VI is calculated by multiplying the average number of mosquitoes collected per trap night by the infection rate. VI is expressed as the average number of infected mosquitoes collected per trap night in the area during the sampling period. In areas with multiple vector species, a VI is calculated for each species, then individual VIs are summed to give a combined estimate of infected vector relative abundance.

Increases in VI reflect increased risk of human disease and serves as a more reliable prediction measure than vector abundance or infection rate alone (Bolling et al. 2009; Jones et al. 2011; Kwan et al. 2012; Colborn et al. 2013). As with other surveillance indicators, the accuracy of the VI depends on the number of trap nights used to estimate abundance and the number of specimens tested to estimate infection rate.

Use of Vector-based Surveillance Indicators: Mosquito-based surveillance indicators have two important roles in arboviral surveillance and response programs. First, they can provide quantifiable thresholds for proactive vector control efforts and public health messaging. By identifying thresholds for vector abundance and infection rates that are below levels associated with disease outbreaks, IVM programs can institute proactive measures to maintain mosquito populations at levels below which virus transmission would be likely. Second, if thresholds related to outbreak levels of transmission can be identified, surveillance can help determine when proactive measures were insufficient to dampen virus amplification and more aggressive measures are needed, such as expanded mosquito control measures and public messaging.

Table 1. Summary of Mosquito-Based Surveillance Indicators

Index	Description	Equation
Vector Abundance	Number of mosquitoes of a particular vector species captured per trap per night	Number of a particular mosquito species captured in a night/Number of traps set up that night
Number of Positive Mosquito Pools	Number of positive mosquito pools detected in a given period of time	Simple count of positive mosquito pools
Percentage of Positive Mosquito Pools	Proportion of positive mosquito pools	Number of positive mosquito pools/Total number of pools tested X 100
Infection Rate	An estimate of the number of mosquitoes infected per 1000 tested	Maximum likelihood estimate (MLE) with bias correction, use links in the footnote. Minimum Infection Rate (MIR) = Number of positive pools/Total number of mosquitoes tested
Vector Index	An estimate of the abundance of infected mosquitoes in an area	$\overline{N}$ = Number of mosquitoes per trap night for a given species $\dot{P}$ = Estimated Infection Rate $\text{Vector Index} = \sum_{i=species} \overline{N}_i \dot{P}_i$

For MLE-based computations use the mosquito surveillance software at
https://www.cdc.gov/westnile/resourcepages/mosqSurvSoft.html

References

Bell JA, Mickelson NJ, Vaughan JA. 2005. West Nile virus in host-seeking mosquitoes within a residential neighborhood in Grand Forks. *North Dakota Vector-Borne Zoonotic Dis.* 5:373

Bolling BG, Barker CM, Moore CG, Pape WJ, Eisen L. 2009. Modeling/GIS, risk assessment, economic impact: Seasonal patterns for entomological measures of risk for exposure to *Culex* vectors and West Nile virus in relation to human disease cases in Northeastern Colorado. *J Med Entomol.* 46:1519-1531.

Colborn, J.M., K.A. Smith, J. Townsend, D. Damian, R.S. Nasci, J.P. Mutebi. 2013. West Nile Virus Outbreak in Phoenix, Arizona—2010: Entomological Observations and Epidemiological Correlations. *J Am Mosq Control Assoc.* 29(2):123-32.

Eldridge BF. 2004. Surveillance for arthropodborne diseases. In Eldridge and Edman eds. Medical Entomology, Kluwer Academic Press, Dordrecht, The Netherlands, pp 645

Godsey MS Jr., Burkhalter K, Young G, Delorey M, Smith K, Townsend J, Levy C, Mutebi JP. 2012. Entomologic investigations during an outbreak of West Nile virus disease in Maricopa County, Arizona, 2010. *Am J Trop Med Hyg.* 87(6):1125-1131.

Gu W, Unnasch TR, Katholi CR, Lampman R, Novak RJ. 2008. Fundamental issues in mosquito surveillance for arboviral transmission. *Trans R Soc Trop Med Hyg.* 102: 817-822.

Gujral IB, Zielinski-Gutierrez EC, LeBailly A, Nasci R. 2007. Behavioral risks for West Nile virus disease, northern Colorado, 2003. *Emerg Infect Dis.* 13(3):419-25.

Holderman CJ, et al. Mosquitoes (Diptera: Culicidae) collected from residential yards and dog kennels in Florida using two aspirators, a sweep net, or a CDC trap. *J Med Ent* 2018;55(1):230-6.

Jones RC, Weaver KN, Smith S, Blanco C, Flores C, Gibbs K, Markowski D, Mutebi JP. 2011. Use of the vector index and geographic information system to prospectively inform West Nile virus interventions. *J Am Mosq Control Assoc.* 27:315-319.

Kwan JL, Park BK, Carpenter TE, Ngo V, Civen R, Reisen WK. 2012. Comparison of enzootic risk measures for predicting West Nile disease, Los Angeles, California, USA, 2004-2010. *Emerg Infect Dis.* 18(8):1298-306.

Nasci RS. 1981. A lightweight battery-powered aspirator for collecting resting mosquitoes in the field. *Mosq News.* 41: 808-811.

Olson JG, Reeves WC, Emmons RW, Milby MM. 1979. Correlation of *Culex tarsalis* population indices with the incidence of St. Louis encephalitis and western equine encephalomyelitis in California. *Am J Trop Med Hyg.* 28: 335-343.

Panella NA, Crockett RJ, Biggerstaff BJ, Komar N. 2011. The Centers for Disease Control and Prevention resting trap: a novel device for collecting resting mosquitoes. *J Am Mosq Control Assoc.* 27(3):323-325.

Turell MJ, Spring AR, Miller MK, Cannon CE. 2002. Effect of holding conditions on the detection of West Nile viral RNA by reverse transcriptase-polymerase chain reaction from mosquito (Diptera: Culicidae) pools. *J Med Entomol.* 39(1):1-3.

Animal-based Surveillance

Bird-based Surveillance

Wild birds are the primary vertebrate hosts of eastern equine encephalitis (EEE) virus and serve as the principal amplification hosts for mosquito infection. EEE epizootics precede human epidemics and in the well-established enzootic EEE virus foci, EEE antibody prevalence among wild birds ranged from 5 to 85% (Elias et al. 2017; Dalrymple et al. 1972; Stamm 1968). However, during epizootics outside the well-established enzootic EEE virus foci, similar antibody prevalence

rates in local wild bird populations were observed (Hayes et al. 1962; Emord and Morris 1984; Stamm 1958; McLean et al. 1985). Some "primary" bird species, typically passerine species, show higher EEE virus reactive antibodies than other bird species and are good sentinels for routine EEE surveillance. Antibody prevalence for primary species during EEE epizootics can range from 40 to 70% (Crans et al. 1994), suggesting intense EEE virus transmission. EEE antibody prevalence in wild bird populations can decline to less than 10% after 3 consecutive non-epizootic years (Hayes et al. 1962; Emord and Morris 1984). Virus activity and antibody seroprevalence for EEE virus 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 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 also provides an effective method for collecting birds (Tsachalidis et al. 2006). Captured birds are bled, banded, and released for possible later recapture to check for seroconversion. Recapture data also gives useful insights on movement, survival, and other population characteristics of the birds. Successful use of this technique requires a labor-intensive sampling effort because of low recapture rates. Because 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 (Smith et al. 1983). This technique requires substantial resources. In addition, it requires highly-trained personnel as well as state and federal collecting permits.

Mortality from EEE virus infection occurs in ring-necked pheasants, emus, and other exotic game bird species (Morris 1988; Saxton-Shaw et al. 2015). Some surveillance programs monitor the morbidity and mortality in captive ring-necked pheasants as sentinels and as an indicator of EEE virus activity.

References

Crans WJ, et al. Eastern equine encephalomyelitis virus in relation to the avian community of a coastal cedar swamp. *J Med Entomol* 1994;31:711–728.

Dalrymple JM, et al. Ecology of arboviruses in a Maryland freshwater swamp. III. Vertebrate hosts. *Am J Epidemiol* 1972;96:129–140.

Elias SP, et al. Seasonal patterns in eastern equine encephalitis virus antibody in songbirds in southern Maine. *Vector Borne Zoonotic Dis* 2017;17:325–330.

Emord DE, Morris CD. Epizootiology of eastern equine encephalomyelitis virus in upstate New York, USA. VI. Antibody prevalence in wild birds during an interepizootic period. *J Med Ent* 1984;21:395–404.

Hayes RO, et al. Entomological aspects of the 1959 outbreak of eastern encephalitis in New Jersey. *Am J Trop Med Hyg* 1962;11:115–121.

McLean RG, et al. Investigations of the vertebrate hosts of eastern equine encephalitis during an epizootic in Michigan, 1980. *Am J Trop Med Hyg* 1985;34:1190–1202.

Morris CD. Eastern equine encephalomyelitis. Monath TP, ed. The Arboviruses: *Epidemiology and Ecology*. Boca Raton (FL): CRC Press; 1988. p. 1–20.

Saxton-Shaw KD, et al. The first outbreak of eastern equine encephalitis in Vermont: Outbreak description and phylogenetic relationships of the virus isolate. *PLoS One* 2015;10:e0128712.

Smith GC, et al. Correlation between human cases and antibody prevalence in house sparrows during a focal outbreak of St. Louis encephalitis in Mississippi, 1979. *Mosq News* 1983;43:322–325.

Stamm DD. Studies on the ecology of equine encephalomyelitis. *Am J Pub Hlth* 1958;48:328–335.

Stamm DD. Arbovirus studies in birds in south Alabama, 1959-1960. *Am J Epidemiol* 1968;87:127137.

Tsachalidis E, et al. The Australian crow trap and the Larsen trap: Their capture success in Greece. Proceedings of the 2006 Naxos International Conference on Sustainable Management and Development of Mountainous and Island Areas. Vol. II. 2006; pp. 325–329.

Live Bird Serology

Chicken flocks are widely used for Western equine encephalitis and St. Louis encephalitis virus surveillance and in some states for EEE virus surveillance. Surveillance for SLE and EEE viruses can take place simultaneously to reduce costs. Like most birds, chickens are susceptible to and can tolerate SLE and EEE virus infections. Chickens, especially older chickens, develop low titer viremia and, therefore, are not likely to contribute to local virus amplification. Chicken flocks can be inexpensively maintained on farms or in urban-suburban locations by residents or health officials. However, it is important to base the choice of locations for the sentinel chickens on historical records of virus activity. Spreading small groups of sentinel chickens throughout the area at risk yields more representative estimates of virus activity. Each spring, 6- to 8-week-old chickens are placed at the selected sentinel sites. Each sentinel site is stocked with 6 to 30 pretested, non-immune, individually banded chickens kept in standard sentinel sheds. Sentinel chickens are bled from the wing vein, the jugular vein, or from the heart weekly, biweekly, or monthly throughout the transmission season. Similar to wild bird surveillance, sentinel chickens were thought to be inappropriate as an early warning system for epidemic activity because the turnaround time from the field to the laboratory results was too long (Morris 1988). Currently, molecular biology-based methods such as RT-PCR and advanced serological methods such as EEE IgM antibody-capture enzyme-linked immunosorbent assay (MAC-ELISA) greatly shorten the turnaround time and in some locations sentinel chicken flocks may be used as early warning systems (Goodman et al. 2015). However, some studies reported failure in some locations (Crans 1986), therefore, use of sentinel chicken flocks needs to be evaluated for each area.

References

Crans WJ. Failure of chickens to act as sentinels during an epizootic of eastern equine encephalitis in southern New Jersey, USA. *J Med Ent* 1986;23(6):626–9.

Goodman CH, et al. Production of a Sindbis/eastern equine encephalitis chimeric virus inactivated cell culture antigen. *J Virol Methods* 2015;223:19–24.

Morris CD. Eastern equine encephalomyelitis. Monath TP, ed. The Arboviruses: Epidemiology and Ecology. Boca Raton (FL): *CRC Press*; 1988. p. 1–20.

Horses and Other Vertebrates

In areas with susceptible horse populations, surveillance for equine cases can provide a sensitive early warning system for EEE outbreaks. Horses are subject to high vector attack rates due to their field exposure. Reports by local veterinarians of equine encephalomyelitis give warning of increased arbovirus activity in an area. This can alert public health officials to investigate the situation. Equine surveillance can be active or passive. 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 accompany samples for laboratory testing if the results are to be useful. Some limitations in using equines include EEE virus immunity from prior vaccination, movement into and out of the surveillance area, and lack of prompt reporting of morbidity or mortality by attending veterinarians.

Several studies report EEE virus antibody-positive sera among populations of free-ranging white-tailed deer, *Odocoileus virginianus*, suggesting white-tailed deer are frequently exposed to EEE virus infections (Hoff et al. 1973; Bigler et al. 1975; Tate et al. 2005; Schmitt et al. 2007). Deer serosurveys have been utilized to study distribution ranges of EEE virus activity especially in northeastern United States (Berl et al. 2013; Mutebi et al. 2011; Mutebi et al. 2015). *Odocoileus virginianus* inhabit a geographically localized home range, often not exceeding a 1.6 km (1 mile) radius, where they both become infected and are harvested (DeNicola et al. 2000; Marchinton and Hirth 1984). Collecting *O. virginianus* blood samples is less labor intensive because of the seasonal deer harvests; samples are collected from the carcasses when hunters bring the harvested deer to the registration station. EEE virus antibody surveillance in harvested *O. virginianus* is a potential tool for EEE surveillance and distribution mapping. Deer serosurveys may be useful for monitoring EEE virus activity but have no predictive value for human infection because deer harvesting occurs in the early fall after the EEE virus transmission season.

Similar studies have been conducted using moose and game birds in the northeastern United States (Mutebi et al. 2012; Lubelczyc et al. 2014; Elias et al. 2017). However, these studies only provide information on distribution ranges of EEE virus activity and cannot be used as early warning systems.

References

Berl E, et al. Serological evidence for eastern equine encephalitis virus activity in white-tailed deer, *Odocoileus virginianus*, in Vermont, 2010. *Am J Trop Med Hyg* 2013;88:103–107.

Bigler WJ, et al. Arbovirus surveillance in Florida: Wild vertebrate studies 1965-1974. *J Wildl Dis* 1975;11:348–356.

DeNicola AJ, et al. Managing white-tailed deer in suburban environments: A technical guide. A publication of Cornell Cooperative Extension, the Wildlife Society—Wildlife Management Working Group, and Northeastern Wildlife Damage Research and Outreach Cooperative. New York (NY). 2000.

Elias SP, et al. Seasonal patterns in eastern equine encephalitis virus antibody in songbirds in southern Maine. *Vector Borne Zoonotic Dis* 2017;17:325–330.

Hoff GL, et al. Arbovirus serology in North Dakota Mule and White-Tailed Deer. *J Wildl Dis* 1973; 9:291–295.

Lubelczyk C, et al. Detection of eastern equine encephalitis virus antibodies in moose (*Alces americana*), Maine, 2010. *Vector Borne Zoonotic Dis* 2014; 14:77–81.

Marchinton RL, Hirth DH. Behavior in Halls LK. White-tailed deer: Ecology and management. Wildlife Management Institute: *Stackpole Books*; 1984. p. 129–168.

Mutebi JP, et al. Using wild white-tailed deer to detect eastern equine encephalitis virus activity in Maine. *Vector Borne Zoonotic Dis* 2011;11:1403–1409.

Mutebi JP, et al. Eastern equine encephalitis in moose (*Alces americanus*) in northeastern Vermont. *J Wildl Dis* 2012;48:1109–1112.

Mutebi JP, et al. Prevalence of eastern equine encephalitis virus antibodies among white-tailed deer populations in Maine. *Vector Borne Zoonotic Dis* 2015;15:210–214.

Schmitt SM, et al. An outbreak of eastern equine encephalitis virus in free-ranging white-tailed deer in Michigan. *J Wildl Dis* 2007;43:635–644.

Tate CM, et al. Eastern equine encephalitis in a free-ranging white-tailed deer (*Odocoileus virginianus*). *J Wildl Dis* 2005;41:241–245.

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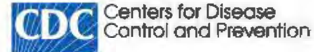
Appendix 1. EEE Virus Testing for for Mosquito Pools (Real-Time RT-PCR)

Last Reviewed: December 5, 2022



Human Disease

Routes of Transmission

EEE virus is maintained in a cycle between *Culiseta melanura* mosquitoes and avian hosts in freshwater hardwood swamps. *Culiseta melanura* is not considered to be an important vector of EEE virus to people because it feeds almost exclusively on birds. Transmission to people requires another mosquito species to create a bridge between infected birds and uninfected mammals, such as people or horses (Morris 1988). Most of the bridge species are within the *Aedes*, *Coquillettidia*, and *Culex* genera. EEE virus has been documented to be transmitted through organ transplantation with one organ donor transmitting the infection to three organ transplant recipients (Pouch et al. 2019). Although not documented, EEE virus likely can be transmitted from person-to-person through blood transfusions.

Clinical Presentation and Evaluation

Most persons infected with EEE virus have no apparent illness (Davis et al. 2008; Calisher 1994). Among those who develop symptoms, the incubation period typically ranges from 4 to 10 days but can be several weeks in people who are immunocompromised (CDC 2021; Sherwood and Oliver 2013).

Symptomatic infection is characterized by fever, chills, malaise, arthralgia, and myalgia (Calisher 1994). Most people recover completely in 1 to 2 weeks unless central nervous system involvement is present. Less than 5% of infected individuals develop meningitis or encephalitis (Morris 1988; Goldfield et al.1968). In infants, neurologic disease often occurs soon after onset; in older children and adults, encephalitis may occur after several days of systemic illness. Signs and symptoms in patients with neuroinvasive disease include headache, confusion, focal neurologic deficits, meningismus, seizures, or coma (Feemster 1938; Przelomski et al.1988; Deresiewicz et al. 1997; Clarke 1961; Letson et al. 1993; Ayers and Feemster 1949). Cerebrospinal fluid (CSF) findings include an initial neutrophil-predominant pleocytosis, shifting to a lymphocyte-predominance, and elevated protein levels; glucose levels are normal (Przelomski et al. 1988; Deresiewicz et al. 1997; Silverman et al. 2013). Neuroimaging shows brain lesions consistent with encephalitis, including neuronal destruction and vasculitis in the cortex, midbrain, and brain stem (Przelomski et al. 1988; Silverman et al. 2013). Magnetic resonance imaging using T2-weighted images often show areas of increased signal in basal ganglia and thalami (Deresiewicz et al. 1997).

Persons aged >50 and <15 years seem to be at greatest risk for developing severe disease when infected with EEE virus. EEE neuroinvasive disease is estimated to have a 30% case fatality rate and results in neurologic sequelae in >50% of survivors (Feemster 1938; Goldfield and Sussman 1968; Deresiewicz et al. 1997; Letson et al. 1993; Ayers and Feemster 1949; Silverman et al. 2013; Gaensbauer et al. 2014). Death typically occurs 2 to 10 days after symptom onset but can occur much later. The neurologic sequelae can range from mild brain dysfunction to severe intellectual impairment, personality disorders, seizures, paralysis, and cranial nerve dysfunction. Many patients with severe sequelae require long-term care and die within a few years.

EEE virus disease should be considered in any person with a febrile or acute neurologic illness with a potential for recent exposure to mosquitoes, organ transplantation, or potentially blood transfusion, particularly during the summer months in areas where virus activity has been reported. In addition to other more common causes of encephalitis and aseptic meningitis (e.g., herpes simplex virus and enteroviruses), other arthropod-borne viruses (e.g., West Nile, La Crosse, St Louis encephalitis, and Powassan viruses) should also be considered in the differential diagnosis of suspected EEE.

References

Ayres JC and Feemster RF. The sequelae of eastern equine encephalomyelitis. *N Engl J Med* 1949;240: 960–962.

Calisher CH. Medically important arboviruses of the United States and Canada. *Clin Microbiol* Rev 1994;7:89–116.

CDC. Eastern equine encephalitis: Clinical Evaluation & Disease. Fort Collins; 2021. https://www.cdc.gov/easternequineencephalitis/healthcare-providers/clinical-evaluation-disease.html.

Clarke DH. Two nonfatal human infections with the virus of eastern encephalitis. *Am J Trop Med Hyg* 1961;10:67–70.

Davis LE, et al. North American encephalitic arboviruses. *Neurol Clin* 2008;26:727–57, ix.

Deresiewicz RL, et al. Clinical and neuroradiographic manifestations of eastern equine encephalitis. *N Engl J Med* 1997;336:1867–1874.

Feemster RF. Outbreak of encephalitis in man due to the eastern virus of equine encephalomyelitis. *Am J Public Health Nations Health* 1938;28:1403–1410.

Gaensbauer JT, et al. Neuroinvasive arboviral disease in the United States: 2003 to 2012. *Pediatrics* 2014;134:e642–e650.

Goldfield M, et al. The 1959 outbreak of eastern encephalitis in New Jersey. The inapparent infection: Disease ratio. *Am J Epidemiol* 1968;87:32–38.

Goldfield M and Sussman O. The 1959 outbreak of eastern encephalitis in New Jersey. I. Introduction and description of outbreak. *Am J Epidemiol* 1968;87:1–10.

Letson GW, et al. Eastern equine encephalitis (EEE): a description of the 1989 outbreak, recent epidemiologic trends, and the association of rainfall with EEE occurrence. *Am J Trop Med Hyg* 1993;49:677–85.

Morris CD. Eastern equine encephalitis. Monath TP, ed. The Arboviruses: Epidemiology and Ecology, Vol. 3. Boca Raton (FL): *CRC Press*; 1988. p. 1–20.

Pouch SM, et al. Transmission of eastern equine encephalitis virus from an organ donor to 3 transplant recipients. *Clin Infect Dis* 2019;69:450–458.

Przelomski MM, et al. Eastern equine encephalitis in Massachusetts: a report of 16 cases, 1970–1984. *Neurology* 1988;38:736–739.

Sherwood JA and Oliver J. Eastern equine encephalitis incubation time periods of 5 and 8 days. *Pediatr Infect Dis J* 2015;34:459–60.

Silverman MA, et al. Eastern equine encephalitis in children, Massachusetts and New Hampshire, USA, 1970–2010. *Emerg Infect Dis* 2013;19:194–201.

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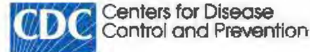

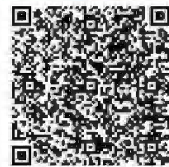

Human Surveillance

Passive Surveillance and Case Investigation

Arboviral diseases are nationally notifiable conditions, and many are explicitly reportable in U.S. states and territories. Most disease cases are reported to public health authorities from public health or commercial laboratories; healthcare providers can also directly submit reports of suspected cases. State and local health departments are responsible for ensuring that reported human disease cases meet the national case definitions. The most recent case definitions for arboviral diseases can be located on the CDC Nationally Notifiable Disease Surveillance System website (https://ndc.services.cdc.gov/conditions/arboviral-diseases-neuroinvasive-and-non-neuroinvasive/). For some arboviruses (e.g., West nile virus (WNV)) presumptive viremic donors are identified through universal screening of the blood supply; case definitions and reporting practices for viremic donors vary by jurisdiction and blood services agency. All identified human disease cases and presumptive viremic blood donors should be investigated promptly. Jurisdictions may choose to interview the patient's healthcare provider, the patient, or both depending on information needs and resources. Whenever possible, the following information should be gathered

- Basic demographic information (e.g., age, sex, race/ethnicity, state, county of residence)
- Clinical syndrome (e.g., asymptomatic blood donor, uncomplicated fever, meningitis, encephalitis, acute flaccid paralysis)
- Illness onset date and/or date of blood donation
- If the patient was hospitalized and if he/she survived or died
- Travel history in the 4 weeks prior to onset
- If the patient was an organ donor or a transplant recipient in the 4 weeks prior to onset
- If the patient was a blood donor or blood transfusion recipient in the 4 weeks prior to onset
- If the patient was pregnant at illness onset
- If the patient is an infant, was he/she breastfed before illness onset

If the patient donated blood, tissues, or organs in the 4 weeks prior to illness onset, immediately inform the blood or tissue bank and appropriate public health authorities. Similarly, any infections temporally associated with blood transfusion or organ transplantation should be reported. Prompt reporting of these cases will facilitate the identification and quarantine of any remaining infected products and the identification of any other exposed recipients so they may be managed appropriately.

Passive surveillance systems are dependent on clinicians considering the diagnosis of an arboviral disease and obtaining the appropriate diagnostic test and reporting of laboratory-confirmed cases to public health authorities. Because of incomplete diagnosis and reporting, the incidence of arboviral diseases is underestimated. Where applicable, reported neuroinvasive disease cases are considered the most accurate indicator of activity in humans because of the substantial associated morbidity. In contrast, reported cases of non-neuroinvasive disease are more likely to be affected by disease awareness and healthcare-seeking behavior in different communities and by the availability and specificity of laboratory tests performed.

Enhanced Surveillance Activities

Enhanced surveillance for human arboviral disease cases should be considered when environmental or human surveillance suggests that an outbreak is suspected or anticipated. Educating healthcare providers and infection control practitioners about the need for arbovirus testing and reporting of all suspected cases could increase the sensitivity of the surveillance system. This might be accomplished by distributing print materials, participating in local hospital meetings and grand rounds, and providing lectures/seminars. Public health agencies should also work to establish guidelines and protocols with local blood collection agencies for reporting viremic blood donors. At the end of the year, an active review of medical records and laboratory results from local hospitals and associated commercial laboratories should be conducted to identify any previously unreported cases. In addition, an active review of appropriate records from blood collection agencies could be conducted to identify any positive donors that were not reported.

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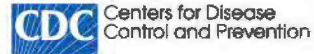

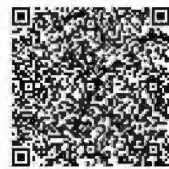

Laboratory Diagnosis and Testing

Laboratory Diagnosis of Human Arboviral Diseases

Laboratory testing for evidence of arboviral diseases typically involves serologic and molecular testing. For several viruses where humans are an amplification host, molecular testing is more specific and can be used to confirm the diagnosis in the first week of illness. For viruses that typically are neuroinvasive, serology is more likely to be used to determine if someone was recently infected.

In most patients, infection with an arbovirus that can cause encephalitis is clinically inapparent or causes a nonspecific viral syndrome. Numerous pathogens cause encephalitis, aseptic meningitis, and febrile disease with similar clinical symptoms and presentations and should be considered in the differential diagnosis. Definitive diagnosis can only be made by laboratory testing using specific reagents. Selection of diagnostic test procedures should take into consideration patient factors (e.g., age, immune status, vaccination history), timing of infection, the range of pathogens in the differential diagnosis, the criteria for classifying a case as confirmed or probable, as well as the capability of the primary and confirming diagnostic laboratories.

Appropriate selection of diagnostic procedures and accurate interpretation of findings requires information describing the patient and the diagnostic specimen. For human specimens, the following data must accompany sera, CSF or tissue specimens for results to be properly interpreted and reported: 1) symptom onset date (when known); 2) date of sample collection; 3) unusual immunological status of patient (e.g., immunosuppression); 4) state and county of residence; 5) travel history (especially in flavivirus-endemic areas); 6) history of prior vaccination (e.g., yellow fever, Japanese encephalitis, or tick-borne encephalitis viruses); and 7) brief clinical summary including clinical diagnosis (e.g., encephalitis, aseptic meningitis). Minimally, onset and sample collection dates are required to perform and interpret initial screening tests. The remaining information is required to evaluate any test results from initial screening. If possible, a convalescent serum sample taken at least 14 days following the acute sample should be obtained to enable confirmation by serological testing.

Human Diagnostic Testing

Eastern equine encephalitis (EEE) virus is a HHS Select Agent, and therefore, subject to strict regulations regarding its possession and use. Those intending to conduct EEE virus testing must be familiar with the complete information and specific guidance found at the Federal Select Agent Program website ***before*** conducting EEE virus testing.

Briefly, samples determined to be positive for EEE virus must be documented and reported to the Federal Select Agent Program via Form 4 (https://www.selectagents.gov/form4.html) within 7 calendar days of identification, and, if not diagnosed at a registered entity, they must then be transferred to a registered Select Agent facility or destroyed with documentation.

Serology

The front-line diagnostic assay for laboratory diagnosis of human EEE virus infection is the IgM antibody assay. Commercially available immunofluorescence assay (IFA) kits to detect IgM or IgG antibodies are often used in public health and other laboratories the United States. In addition, IgM and IgG assays developed at CDC are available in both

ELISA and microsphere (IgM) immunoassay (MIA; Basile et al. 2013) formats; protocols and limited supplies of reagents are available from CDC's DVBD Diagnostic Laboratory. CDC will provide positive controls and limited reagents considering commercial sources are available to state public health labs.

Because the IgM and IgG assays can be positive due to non-specific reactivity or rarely cross-reactivity (e.g., EEE virus is the only virus in the EEE antigenic complex in the United States, but low-level cross-reactivity might occur with other alphaviruses), they should be viewed as a presumptive positive. For a case to be considered confirmed, serum samples that are antibody-positive on initial testing should be evaluated by a more specific assay. Currently, the plaque reduction neutralization test (PRNT) is recommended for confirming IgM serological results. Although EEE virus is a rare cause of arboviral encephalitis in the United States, several other arboviral encephalitides are present in the United States and in other regions of the world. Specimens submitted for EEE virus testing should also be tested by ELISA and PRNT against other arboviruses known to be active or present in the area or in the region to where the patient traveled.

Virus Detection Assays

Numerous procedures have been developed for detecting viable EEE virus, EEE virus antigen, or EEE virus RNA in human diagnostic samples, many of which have been adapted to detecting EEE virus in other vertebrates and in mosquito samples. These procedures vary in their sensitivity, specificity, and time required to conduct the test. Among the most sensitive procedures for detecting EEE virus in samples are those using RT-PCR to detect EEE virus RNA in human CSF, serum, and other tissues. Real-time RT-PCR, standard RT-PCR, and nucleic acid sequence-based amplification (NASBA) amplification methods have been developed and validated for specific human diagnostic applications (Lambert et al. 2003); however, no commercially-produced or FDA-approved molecular EEE virus diagnostic tests are available.

EEE virus presence can be demonstrated by isolation of viable virus from samples taken from clinically ill patients. Appropriate samples include CSF, serum samples obtained very early in infection, and brain tissue taken at biopsy or postmortem. Virus isolation should be performed in known susceptible mammalian (e.g., Vero) or mosquito cell lines (e.g., C6/36). Mosquito origin cells may not show obvious cytopathic effect and must be screened by immunofluorescence or RT-PCR. Confirmation of virus isolate identity can be accomplished by indirect immunofluorescence assay (IFA) using virus-specific monoclonal antibodies (MAbs) or nucleic acid detection (e.g., RT-PCR, real-time RT-PCR, or sequencing). The IFA using well-defined murine MAbs is an efficient, economical, and rapid method to identify alphaviruses isolated in cell culture. Incorporating MAbs specific for other arboviruses known to circulate in various regions will increase the rapid diagnostic capacities of state and local laboratories. Nucleic acid detection methods include real-time and standard RT-PCR methods.

Immunohistochemistry (IHC) using virus-specific MAbs on tissue has been useful in identifying both human and veterinary cases of EEE virus infection. In suspected fatal cases, IHC should be performed on formalin fixed autopsy, biopsy, and necropsy material, ideally collected from multiple anatomic regions of the brain, including the brainstem, midbrain, and cortex.

References

Basile AJ, et al. Multiplex microsphere immunoassays for the detection of IgM and IgG to arboviral diseases. *PLoS One* 2013;8:e75670.

Lambert AJ, et al. Detection of North American eastern and western equine encephalitis viruses by nucleic acid amplification assays. *J Clin Microbiol* 2003; 41:379 –385.

Resources for Human Diagnostic Laboratories

Clinical Laboratory Improvements Amendments (CLIA) certification: To maintain certification, CLIA recommendations for performing and interpreting human diagnostic tests should be followed. Laboratories performing arboviral serology or RNA-detection testing are invited to participate in the annual proficiency testing that is available from CDC's Division of Vector-Borne Diseases (DVBD) in Fort Collins, CO. To obtain additional information about the proficiency testing program and about training in arbovirus diagnostic procedures, contact the DVBD by phone: 970-261-6400 or email: dvbid2@cdc.gov.

Biocontainment: Containment specifications are available in the CDC/National Institutes of Health publication *Biosafety in Microbiological and Biomedical Laboratories (BMBL 6)*. This document can be found online at: https://www.cdc.gov/labs/BMBL.html.

Shipping of diagnostic samples and agents. Shipping and transport of clinical specimens should follow current International Air Transport Association (IATA) and Department of Commerce recommendations. For more information, visit the IATA dangerous goods Web site at: http://www.iata.org/publications/dgr/Pages/index.aspx , and the USDA Animal and Plant Health. Inspection Service (APHIS), National Center for Imports and Exports website: https://www.aphis.usda.gov/aphis/ourfocus/importexport .

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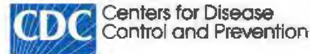

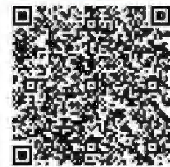

Laboratory Testing of Vectors

Identification and Pooling

Mosquitoes should be identified to species or lowest taxonomic unit. Specimens are placed into pools of 50 specimens or less based on species, sex, location, trap-type, and date of collection. Larger pool sizes can be used in some assays with loss of sensitivity (Sutherland and Nasci 2007). If resources are limited, testing of mosquitoes for surveillance purposes can be limited to the primary vector species.

Homogenizing and Centrifugation

After adding an appropriate homogenization buffer (cell culture medium, viral transport medium, PBS), mosquito pools can be macerated or ground by a variety of techniques including mortar and pestle, vortexing sealed tubes containing one or more copper clad BBs, or by use of tissue homogenizing apparatuses that are commercially available. After grinding, samples are centrifuged, and an aliquot is removed for testing. Because mosquito pools may contain arboviruses and other pathogenic viruses which may be aerosolized during processing, laboratory staff should take appropriate safety precautions including use of a Class II Type A biological safety cabinet, appropriate personal protective equipment (PPE) and biosafety practices.

Virus Detection

EEE virus is an HHS Select Agent, and therefore, subject to strict regulations regarding its possession and use. Those intending to conduct EEE virus testing must be familiar with the complete information and specific guidance found at the Federal Select Agent Program website ***before*** conducting EEE virus testing.

Briefly, samples determined to be positive for EEE virus must be documented and reported to the Federal Select Agent Program via Form 4 (https://www.selectagents.gov/form4.html) within 7 calendar days of identification, and, if not diagnosed at a registered entity, they much then be transferred to a registered Select Agent facility or destroyed.

Multiple diagnostic methods can be used to identify EEE virus in mosquitoes including virus isolation, molecular assays, or immunoassays. Virus isolation in Vero cell culture (Armstrong et al. 2011) remains the standard for confirmation of positive pools but is time consuming and requires specialized laboratory facilities. For virus isolation, mosquito pool homogenates are added to Vero cell cultures, monitored for cytopathic effect, and identified using appropriate diagnostic assays. Aliquots are stored at -70°C to retain virus viability for future testing. Vero cell culture has an additional benefit of detecting other viruses in the mosquitoes, a feature lost using test procedures that target virus-specific nucleotide sequence or proteins (Andreadis et al. 1998; Andreadis et al. 2004). Molecular assays detect viral RNA or nucleic acids in mosquito pools quickly, with high sensitivity and specificity, and do not require cold chain or high levels of biocontainment (Lanciotti et al. 2000; Callahan et al. 2001; Lambert et al. 2003; Armstrong et al. 2012). Methods include rapid molecular assays, RT-PCR, and other nucleic acid amplification tests (e.g., multi-target RT-PCR). Assays may use different primer sets for detection and confirmation of virus in mosquito pools (Lambert et al. 2003). Antigen detection assays are less sensitive than molecular assays, but for some arboviruses (e.g., WNV) can provide valuable infection rate data when employed consistently in a mosquito surveillance program. For additional details see: Human Diagnostic Detection and Annex 1 Real Time RT-PCR Protocol for Mosquito Pools.

References

Andreadis TG, et al. Multiple isolations of eastern equine encephalitis and highlands J viruses from mosquitoes (Diptera: Culicidae) during a 1996 epizootic in southeastern Connecticut. *J Med Entomol* 1998;35:296–302.

Andreadis TG, et al. Epidemiology of West Nile virus in Connecticut, USA: A five-year analysis of mosquito data, 1999-2003. *Vector Borne Zoonotic Dis* 2004;4:360–378.

Armstrong PM, et al. Detection of infectious virus from field-collected mosquitoes by Vero cell culture assay. *J Vis Exp* 2011;pii2889.

Armstrong PM, et al. Development of a multi-target TaqMan assay to detect eastern equine encephalitis virus variants in mosquitoes. *Vector Borne Zoonotic Dis* 2012;12(10):872–876.

Callahan JD, et al. Development and evaluation of serotype- and group-specific fluorogenic reverse transcriptase PCR (TaqMan) assays for dengue virus. *J Clin Microbiol* 2001;39:4119–4124.

Lambert AJ, et al. Detection of North American eastern and western equine encephalitis viruses by nucleic acid amplification assays *J Clin Microbiol* 2003 41:379–385.

Lanciotti RS, et al. Rapid detection of West Nile virus from human clinical specimens, field-collected mosquitoes, and avian samples by a TaqMan reverse transcriptase-PCR assay. *J Clin Microbiol* 2000;38:4066–4071.

Sutherland GL, Nasci RS. Detection of West Nile virus in large pools of mosquitoes. *J Am Mosq Control Assoc* 2007;23:389–395.

Laboratory Testing of Non-human Vertebrates

The choice of laboratory diagnostic tests depends on the needs, approach, and surveillance capability of a given health agency. Tests include antibody-capture ELISA, complement fixation (CF), hemagglutination inhibition (HI), and plaque reduction neutralization test (PRNT). However, few reagents are commercially available for domestic or wildlife IgM antibodies for antibody capture ELISAs. Many public health laboratories will therefore use PRNT because these are not dependent on species specific antibodies.

Serology

The same serologic techniques applied to clinically ill animals may also be used for healthy subjects for vertebrate serosurveys or for healthy sentinel animals serially-sampled as sentinels. As with human diagnostic samples, serologic results from non-human vertebrates must be interpreted with caution due to potential cross-reactivity. Cross reactions may occur between EEE and WEE antibodies in the CF and HI tests. Vaccination for EEE can also cause positive PRNT, HI, CF, and possibly IgM test results.

Specimens from horses and other domestic animals can be tested through diagnostic laboratories including U.S. Department of Agriculture's (USDA) Animal and Plant Health Inspection Service's (APHIS) National Veterinary Services Laboratory (NVSL) in Ames, Iowa. Testing can take up to several weeks to complete depending upon the type of sample submitted and the testing protocol required to obtain a definitive result. Details on the diagnostic criteria for EEE for can be found at: https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/animal-disease-information/equine/eee-wee-vee/equine-encephalitis

For additional details see: Human Laboratory Diagnosis and Testing.

Virus Detection

Methods for virus detection, isolation, and identification are the same as described for human and mosquito diagnostics. The most commonly used methods to detect EEE virus or viral RNA in animal populations are immunoassays, virus isolation, and molecular tests. Specimens typically are tissues or fluids from acutely ill or dead animals. Virus detection in apparently healthy animals is very low-yield and inefficient, and therefore not cost-effective, and should not be

considered for routine surveillance programs. Some animals have few tissues with detectable virus particles or viral RNA at necropsy, such as horses. Others, such as certain bird species, may have fulminant infections with high viral loads in almost every tissue.

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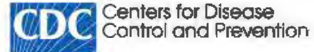

Mosquitoes

Objectives of Surveillance

Objectives of Arboviral Surveillance

Arboviral surveillance consists of two distinct, but complementary activities. Epidemiological surveillance measures human disease to quantify disease burden, detects early signs of an outbreak, and identifies information needed for timely responses, including seasonal, geographic, and demographic patterns in human morbidity and mortality. In addition to monitoring disease burden and distribution, epidemiological surveillance has been instrumental in characterizing clinical disease presentation and disease outcome, as well as identifying high-risk populations and human factors associated with serious disease. Epidemiological surveillance has also detected and quantified alternative routes of transmission to humans, such as contaminated blood donations and organ transplantation. Environmental surveillance monitors local mosquito populations, virus activity in vectors and non-human vertebrate hosts, and other relevant environmental parameters to predict human risk and prevent outbreaks of arboviral disease in humans. Epidemiological and environmental surveillance for arboviruses is facilitated by ArboNET, the national arbovirus surveillance system. ArboNET was developed in 2000 as a comprehensive surveillance data capture platform to monitor West Nile virus (WNV) infections in humans, mosquitoes, birds, and other animals. This comprehensive approach was essential to tracking the progression of WNV as it spread and became established across the United States, and it remains a significant source of data on the epidemiology and ecology of WNV. Since 2003, ArboNET has also collected data on other domestic and exotic arboviruses of public health significance.

In the absence of effective human vaccines for most domestic arboviruses, preventing arboviral disease in humans primarily depends on measures to keep infected vectors from biting people. A principal objective of environmental surveillance is to quantify the intensity of virus transmission in a region and provide a predictive index of human infection risk. This risk prediction, along with information about the local conditions and habitats, and virus-vector interactions that impact vector abundance and infection, can be used to inform an Integrated Vector Management (IVM) program and decisions about implementing interventions to control mosquitoes and to subsequently prevent disease.

Though epidemiological surveillance is essential for understanding arboviral disease burden, utilizing human case surveillance by itself is insufficient for predicting outbreaks. Outbreaks can develop quickly, with most human cases occurring over a few weeks during the peak of transmission. The time from human infection to onset of symptoms to diagnosis and reporting can be several weeks or longer. As a result, human case reports typically lag well behind the transmission from vectors that initiated the infection. Cases in non-human vertebrate hosts are often the first indicator of local arboviral activity. Comparing infection prevalence in vectors and non-human vertebrate cases with historical environmental and epidemiological data can help identify conditions associated with human risk 2 to 4 weeks before human disease onset. This provides additional lead time for critical vector control interventions and public education programs to be put in place. The following sections describe the elements of epidemiological and environmental arboviral surveillance and how they may be used to monitor and predict risk and to trigger interventions.

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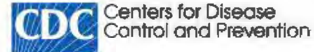

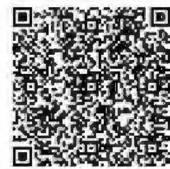

Mosquitoes

Prevention and Control: Community Engagement

Individual-Level Actions to Reduce Risk

Without an effective vaccine for people, the best way to prevent mosquito-borne disease is by preventing mosquito bites. This can be accomplished through community-based IVM programs and by personal protection behaviors, such as

- **Mosquito-avoidance.** Health officials may recommend residents avoid outdoor activities when high virus activity levels have been detected or when mosquitoes are most active.
- **Use of personal insect repellents.** CDC recommends using EPA-registered insect repellents or covering up with long-sleeved shirts and long pants when outside.
- **Removal of residential mosquito sources.** Once a week, residents should empty, cover, or throw out items that hold water, such as tires, buckets, planters, toys, pools, birdbaths, flowerpots, or trash containers.

Jurisdictions can promote individual and community-based prevention measures through public education and risk communication activities. Messages should acknowledge the seriousness of the disease without promoting undue fear or panic in the target population. Fear-driven messages may heighten the powerlessness people express in dealing with vector-borne diseases. Messages should be clear and consistent with the recommendations of coordinating agencies and include a call to action. Use plain language and adapt materials for lower literacy and non-English speaking audiences.

People can further reduce their risk of mosquito bites by not going outdoors when mosquitoes are biting and following recommendations to avoid outdoor activity when and where high virus activity levels have been detected are a component of prevention programs. Recommendations to avoid being outdoors during peak mosquito biting times may conflict with neighborhood social patterns, community events, people's jobs or the practices of persons without air-conditioning. It is important to communicate when the important mosquito vectors are most active. Emphasize that insect repellent use is protective and should be used when outdoors, particularly during the prime mosquito-biting hours.

Additional information about personal insect repellents, including permethrin, can be found at https://www.cdc.gov/mosquitoes/mosquito-bites/prevent-mosquito-bites.html.

Information for individuals on control mosquitoes around their home can be found at https://www.cdc.gov/mosquitoes/mosquito-control/athome/outside-your-home/index.html.

Prevention Strategies for High-Risk Groups

Audience members have different disease-related concerns and motivations for action. Proper message targeting (including use of plain language) permits better use of limited communication and prevention resources. The following are some population segments that require specific targeting. See Human Disease Section for additional information about risk groups that should be targeted.

Persons with Outdoor Exposure. People who engage in extensive outdoor work or recreational activities are at greater risk of being bitten by mosquitoes. Messages for these individuals should encourage use of insect repellent and long-sleeved shirts and pants. Local spokespersons (e.g., union officials, job-site supervisors, golf pros, sports organizations,

lawn care professionals, public works officials, gardening experts) may be useful collaborators. Place messages in locations where people engage in outdoor activities (e.g., parks, golf courses, hiking trails).

People Experiencing Homelessness. Extensive outdoor exposure and limited financial resources in this group present special challenges. Application of insect repellents to exposed skin and clothing may be most appropriate prevention measures for this population. Work with social service groups in your area to educate and provide insect repellents to this population segment.

Residences Lacking Window and Door Screens. The absence of intact window/door screens might increase exposure to mosquito bites. Encourage residents to consistently use screened windows and doors to keep mosquitoes outside. Focus attention on the need to repair screens and provide access to resources to do so. Partner with community organizations that can assist with installing or repairing screens for older persons or others with financial or physical barriers.

Older Adults. For many mosquito-borne diseases, older adults are at greater risk for serious disease. Messages on mosquito avoidance, insect repellent use, and weekly removal of standing water where mosquitoes lay eggs around the home should be shared with this audience.

Communication And Community Engagement

At the community level, advocating for organized mosquito abatement and participating in community mobilization projects to address sources of mosquitoes such as trash, standing water, or untreated swimming pools are activities that can help protect individuals and at-risk groups.

Providing clear messages and understandable concepts promotes community understanding and acceptance. The following provides a description of selected best practices for reaching high-risk groups, offers suggestions for cultivating partnerships with media and communities, and provides select outreach measures for mobilizing communities.

Communicating about Vector Control. Public understanding and acceptance of emergency adult mosquito control operations using insecticides is critical to its success, especially where these measures are unfamiliar. Questions about the products being used, their safety, and their effects on the environment are common. Improved communication about surveillance and how decisions to use mosquito adulticides are made may help residents weigh the risks and benefits of control. When possible, provide detailed information regarding the schedule for adulticiding through newspapers, radio, government-access television, the internet, recorded phone messages, social media, or other means your agency uses to successfully communicate with its constituencies.

Community Mobilization and Outreach. Community mobilization can improve education and help achieve behavior change goals. Promote the concept that health departments and mosquito control programs require community assistance to reduce mosquito-borne disease risk. Leverage online platforms to further disseminate your messages.

A community task force that addresses civic, business, public health, and environmental concerns can be valuable in achieving buy-in from various segments of the community, and in developing common messages. Community mobilization activities can include clean-up days to get rid of mosquito habitats (e.g., tires, trash). Effective community outreach also involves presenting messages in person, involving citizens in prevention and control activities, and using traditional and social media outreach. Hearing the message of personal prevention from community leaders can validate the importance of the disease and serve as a community call to action. Health promotion events and activities reinforce the importance of prevention and control in a community setting.

Partnership with Media and the Community. It can be beneficial to cultivate relationships with the media (e.g., radio, TV, newspaper, web-based news outlets) prior to an outbreak. Obtain media training for at least one member of your staff and designate that individual as the organization's spokesperson. Develop key messages and a communication plan, including press releases, prior to developing products. Many communities have heard mosquito prevention and control messages repeated for several years. Getting the public's attention when risk levels increase can be a challenge. Therefore, evaluate and update mosquito bite prevention messages annually and test new messages with different population segments to evaluate effectiveness. Develop partnerships with agencies and organizations that have relationships with populations at higher risk (such as persons over 50 years of age) or are recognized as community leaders (e.g., churches, service groups). Working through sources trusted by the priority audience can heighten the credibility of and attention to messages. Partnerships with businesses that sell materials to fix or install window screens or that sell insect repellent may be useful in some settings (e.g., local hardware stores, grocery stores).

Social Media. A majority of Americans use social media which can be an inexpensive and rapid method for disseminating information to the community. Outreach can be conducted using Twitter, Facebook, YouTube, blogs, and other websites that may reach constituents less connected to more traditional media sources. Using images or videos in your posts make them more attention grabbing. It is also best practice to include a call-to-action people can take. Provide links that direct users to webpages or other resources with more complete information.

Online Resources. The Internet has become a primary source of health information for most Americans. Encourage constituents to seek advice from credible sources. Make sure local public health agency websites are clear, accurate, and up to date. Useful information is available from a number of resources:

- The CDC web pages are updated frequently to reflect new findings and recommendations. Materials on the CDC web site are in the public domain and serve as a resource for state and local health departments and other organizations.
- CDC staff can provide technical assistance in the development of audience research and strategies for public education and community outreach. Contact CDC, Division of Vector-Borne Diseases' health communications staff in Fort Collins, CO at 970-221-6400.
- The U.S. Environmental Protection Agency (EPA) is the government's regulatory agency for insecticide and insect repellent use, safety, and effectiveness. Information about mosquito control insecticides and repellents is available at https://www.epa.gov/mosquitocontrol. These include guidance for using insect repellents safely and a search tool to assist in finding a repellent that is right for you (https://www.epa.gov/insect-repellents/find-repellent-right-you#searchform), which allows the user to examine the protection time afforded by registered insect repellents containing various concentrations of the active ingredients.

There are several non-governmental organizations that have developed useful tools and information that can be adapted for local needs. Examples include: the American Mosquito Control Association (https://www.mosquito.org/default.aspx) and the National Pesticide Information Center (NPIC) (www.npic.orst.edu).

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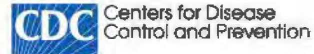

Mosquitoes

Prevention and Control: Integrated Vector Management

Integrated Vector Management

Prevention and control of arboviral diseases is accomplished most effectively through a comprehensive, Integrated Vector Management (IVM) program applying the principles of Integrated Pest Management. IVM is based on an understanding of the underlying biology of the arbovirus transmission system and utilizes regular monitoring of vector mosquito populations and arboviral activity levels to determine if, when, and where interventions are needed to keep mosquito numbers below levels which produce risk of human disease, and to respond appropriately to reduce risk when it exceeds acceptable levels.

Operationally, IVM is anchored by a monitoring program providing data that describe

- Conditions and habitats that produce vector mosquitoes
- Abundance of those mosquitoes over the course of a season
- Arboviral transmission activity levels expressed as infection rate in mosquito vectors
- Parameters that influence local mosquito populations and virus transmission

These data inform decisions about implementing mosquito control activities appropriate to the situation, such as

- Source reduction through habitat modification
- Larval mosquito control using the appropriate methods for the habitat
- Adult mosquito control using pesticides applied from trucks or aircraft when established thresholds have been exceeded
- Community education efforts related to risk levels and intervention activities

Monitoring also provides quality control for the program, allowing evaluation of the effectiveness of larval and adult control efforts, and causes of control failures (e.g., undetected larval sources, pesticide resistance, equipment failure).

Mosquito Control Activities

Mosquito control tools target mosquitoes at the adult or immature stage depending on program objectives. Multiple species are involved in eastern equine encephalitis (EEE) virus transmission, and different populations of a single species may vary their activity based on environmental conditions. The decision to conduct mosquito control activities is based on mosquito and meteorological surveillance, established local thresholds and triggers (mosquito, human, and non-human animal), and insecticide resistance status of the target species (Table 1). Mosquito control professionals should have detailed knowledge of the local mosquitoes involved in EEE virus transmission to prevent and control disease. Programs should use pesticides and other control tools registered by the U.S. Environmental Protection Agency (EPA) in compliance with label instructions and any local, state, and federal laws regulating their use.

Table 1. Summary of Mosquito Control by Life-stage, Method, and Objective

Life Stage	Method	Objective	Example	Notes
Larvae	Environmental management (modification/manipulation)	Eliminate or disrupt larval aquatic habitats to reduce adult mosquitoes	• Wetland management • Biological control	May be prohibited or logistically unfeasible
Larvae	Larvicide application to aquatic habitats	Kill immature mosquitoes to reduce adult populations	• Insect Growth Regulators (e.g., pyriproxyfen, methoprene) • Microbial products (e.g., Bti/Bs/Spinosad) • Oils and films	Limited data on efficacy. *Culiseta melanura* habitats may be inaccessible by ground/aerial application
Adult	Ultra-low volume (ULV) insecticides	Reduce the adult mosquito population active at the time of treatment	• Space Spray	Targets mosquitoes active at the time of application
Adult	Residual adulticides	Residual control of mosquitoes	• Residual treatments to surfaces • Barrier treatments to vegetation	Targets resting mosquitoes
Adult	Traps and baits	Attract and kill adult mosquitoes	• Attractive targeted sugar bait (ATSB)	Host-seeking, sugar-seeking or ovipositing mosquitoes

http://www.epa.gov/mosquitocontrol

Larval Mosquito Control

The objective of larval mosquito control is to reduce immature mosquito populations before they emerge as adults. This can be an efficient method of managing mosquitoes where larval sites are accessible, but habitats of EEE virus vectors are often hard to find and labor-intensive to treat. Few studies have shown efficacy of larval control methods against EEE virus vectors.

Culiseta melanura larvae develop in crypts filled with water in swamp and bog habitats. A single study showed aerial application of methoprene penetrated larval crypts and had 81% efficacy (emergence inhibition) over 5-weeks post-treatment (Woodrow et al. 1995). Temephos was also evaluated and not detected in the larval habitats (crypts) of *Cs. melanura*. Although not evaluated yet for EEE, aerial or ULV *Bacillus thuringiensis israelensis* (Bti) water-dispersible granules can penetrate foliage and water in covered areas to control other mosquitoes that occur in cryptic larval habitats (e.g., *Aedes aegypti*, *Culex quinquefasciatus*) (Pruszinski et al. 2017). These delivery techniques may be also useful against *Cs. melanura* and the larval habitats of epizootic bridge vectors. Although further studies are needed on the efficacy and implementation of larval control of EEE virus, applying a larvicide at the same time as an adulticide application to reduce adult mosquito populations may help prevent population rebound due to newly emerged adults and mosquitoes not active at the time of application.

Larvicides (and pupacides) are applied directly to water sources or placed in areas where flooding is expected to target the aquatic habitats of vector species. Larvicide can be applied by ground or aerial dispersal methods. For small aquatic larval sites or areas that cannot be reached by vehicles, backpack sprayers and dusters are used to apply liquid, granules, or pellets. Formulations can be short-acting (up to 2 weeks) or extended-release products (lasting more than 1 month). Larvicides may kill on contact through ingestion, or act as stomach poisons or growth regulators. Information on pesticides for larval mosquito control is available from the U.S. EPA (http://www.epa.gov/mosquitocontrol/controlling-mosquitoes-larval-stage).

Adult Mosquito Control

Adult mosquito control aims to reduce the abundance of biting, infected adult mosquitoes to prevent them from transmitting arboviruses to humans and to break the mosquito-host transmission cycle. Where populations are increasing above acceptable levels, adulticides are used to reduce vectors. Vector mitigation strategies should be applied quickly once arboviral activity is detected and be targeted to the local EEE virus epizootic and enzootic vectors. Programs should use pesticides registered by EPA for this purpose (http://www.epa.gov/mosquitocontrol/controlling-adult-mosquitoes).

Adulticides can reduce the numbers of adult mosquito vectors for EEE virus, but not enough cases occur annually to demonstrate clear impact on EEE virus transmission to humans. Indicators of high transmission risk are used to decide when to apply adulticides and often by the time aerial applications occur, transmission to humans has already occurred. Also, due to the epidemic nature of this disease, untreated areas relevant for comparison might not be available, which limits the ability to make conclusions about the efficacy of using adulticides to reduce disease (Grady et al. 1978).

Adulticiding can be conducted from the ground with backpack spray equipment, truck-mounted equipment, or by air with fixed-wing or rotary-wing applications. Types of treatment include space-spray (e.g., ULV) adulticides and residual treatments.

- Space-spray and ULV treatments rely on mosquitoes and insecticide droplets coming into direct contact in the air column. These are temporary measures to reduce the mosquito population active at the time of treatment (Lloyd et al. 2018). ULV formulations applied in small volumes prevent deposition and enhance degradation of the active ingredients in the environment (Bonds 2012). Mosquitoes not active at the time of application are not exposed. Because there is little to no deposition of insecticide, no residual control of mosquitoes occurs. As a result, multiple applications may be needed for sustained control (Andis et al. 1987).
- Long-lasting adulticides, also called residual or barrier treatments, can be applied to surfaces and to vegetation. To be effective, the mosquito must land on the treated surface and directly contact the insecticide. This type of application targets the resting mosquito population and is typically used in urban pest management and residential properties (Lloyd et al. 2018).
- Other methods of control: Traps and baits have been proposed to control mosquitoes (e.g., *Ae. aegypti, Ae. albopictus)* but few studies have been conducted for vectors of EEE virus. A single study on attractive targeted sugar baits (ATSB), which attract and kill sugar feeding mosquitoes, found reductions in adult *Cs. melanura* populations in the 2-weeks post-treatment; however, the study design, number of mosquitoes trapped, and background insecticide used in the study limit the conclusions. At present, more evidence is needed before for broad scale use can be recommended. (Qualls et al. 2014).

References

Andis MD, et al. Strategies for the emergency control of arboviral epidemics in New Orleans. *J Am Mosq Control Assoc* 1987;3:125–130.

Bonds JA. Ultra-low-volume space sprays in mosquito control: a critical review. *Med Vet Entomol* 2012;26:121–130.

Grady GF, et al. Eastern equine encephalitis in Massachusetts, 1957-1976: A prospective study centered upon analyses of mosquitoes. *Am J Epidemiol* 1978;107:170–178.

Lloyd AM, Connelly CR, and Carlson DB (Eds.). Florida Coordinating Council on Mosquito Control. Florida Mosquito Control: The state of the mission as defined by mosquito controllers, regulators, and environmental managers. Vero Beach (FL): University of Florida, Institute of Food and Agricultural Sciences, Florida Medical Entomology Laboratory; 2018. https://fmel.ifas.ufl.edu/media/fmelifasufledu/7-15-2018-white-paper.pdf

Pruszynski CA, Hribar LJ, Mickle R, Leal AL. A large scale biorational approach using *Bacillus thuringiensis israelensis* (Strain AM65-52) for managing *Aedes aegypti* populations to prevent dengue, chikungunya and Zika transmission. *PLoS One* 2017;12:e0170079.

Qualls WA, et al. Evaluation of attractive toxic sugar bait (ATSB)—Barrier for control of vector and nuisance mosquitoes and its effect on non-target organisms in sub-tropical environments in Florida. *Acta Tropica* 2014;131:104–110.

Woodrow RJ, et al. Field trials with methoprene, temephos, and *Bacillus thuringiensis* serovar *israelensis* for the control of larval *Culiseta melanura*. *J Am Mosq Control Assoc* 1995;11:424–427.

Safety and Quality of Vector Control Pesticides and Practices

Insecticides to control larval and adult mosquitoes are registered specifically for that use by the EPA. Instructions provided on the product labels prescribe the required application and use parameters and must be carefully followed. Properly applied, these products do not negatively affect human health or the environment. In persons living in treated areas, ULV application of mosquito control adulticides does not produce any detectable biological changes indicating exposure or increase asthma or other adverse health events (Currier et al. 2005; Duprey et al. 2008; Karpati et al. 2004). The morbidity and mortality from arboviruses demonstrably exceed the risks from mosquito control practices (Davis and Peterson 2008; Macedo et al. 2010; Peterson et al. 2006).

Legal Action to Achieve Access or Control

Individually owned private properties may be major sources of mosquito production. Examples include accumulations of discarded tires or other trash, neglected swimming pools, and similar water features that become stagnant and produce mosquitoes. Local public health statutes or public nuisance regulations may be employed to gain access for surveillance and control or to require the property owner to mitigate the problem. Executing such legal actions may be a prolonged process during which adult mosquitoes are continuously produced. Proactive communication with residents and public education programs may alleviate the need to use legal actions. However, legal efforts may be required to eliminate persistent mosquito production sites.

Quality of Control

Pesticide products and application procedures (for both larval and adult control) must periodically be evaluated to ensure an effective rate of application is being used and that the desired degree of control is obtained. Application procedures should be evaluated regularly (minimally once each season) to assure equipment is functioning properly to deliver the correct dosages and droplet parameters and to determine appropriate label rates to use locally. Finally, mosquito populations should routinely be evaluated to ensure insecticide resistance is not emerging.

Records

Surveillance data describing vector sources, abundance and infection rates, records of control efforts (e.g., source reduction, larvicide applications, adulticide applications), and quality control data must be maintained and used to evaluate IVM needs and performance. Long-term data are essential to track trends and to evaluate levels of risk.

Insecticide Resistance Management

For vector control to be effective, mosquitoes must be susceptible to the insecticide selected for use. In order to delay or prevent the development of insecticide resistance in vector populations, IVM programs should include a resistance management component (Lloyd et al. 2018). This should include routine monitoring of the status of resistance in the target populations to

- Provide baseline data for program planning and pesticide selection before the start of control operations
- Detect resistance at an early stage so that timely management can be implemented
- Continuously monitor the effect of control strategies on insecticide resistance, and determine potential causes for control failures, should they occur

Insecticide resistance may be monitored using bioassays in larvae or adult mosquitoes (Brogden and McAllister 1998). The CDC bottle bioassay is a simple, rapid, and economical tool to detect insecticide resistance by determining the time taken for a pesticide active ingredient to kill mosquito vectors. The results can help guide the choice of insecticide used for spraying. The CDC bottle bioassay can be used as part of a broader insecticide resistance monitoring program, which may include field cage tests and biochemical and molecular methods. A practical laboratory manual for the CDC bottle bioassay is available online https://www.cdc.gov/mosquitoes/mosquito-control/professionals/cdc-bottle-bioassay.html. For additional information, contact CDC at USBottleAssayKit@cdc.gov.

The IVM program should include options for managing resistance that are appropriate for local conditions. The techniques regularly used include the following:

- Management by moderation. Prevent onset of insecticide resistance by reducing overall chemical use or persistence by
    - Using doses no lower than the lowest label rate to avoid genetic selection
    - Using chemicals of short environmental persistence and avoiding slow-release formulations that increase selection for resistance
    - Avoiding use of the same class of insecticide to control adult and immature stages
    - Applying locally; many districts treat only hot spots and use area-wide treatments only during public health alerts or outbreaks
    - Using less frequent applications; leaving generations, population segments, or areas untreated (when appropriate)
    - Establishing higher thresholds for mosquito mitigation with insecticides, except during public health alerts or outbreaks.
- Management by continued suppression. This strategy is used in regions of high value or persistent high risk (e.g., heavily populated regions or locations with recurring outbreaks) where mosquitoes must be kept at very low densities. It involves the application of dosages within label rates but sufficiently high to be lethal to heterozygous individuals that are partially resistant. If the heterozygous individuals are killed, resistance will be slow to emerge. This method should not be used if any significant portion of the population in question is fully resistant. Another approach more commonly used is the addition of synergists that inhibit existing detoxification enzymes and thus eliminate the competitive advantage of these individuals. Commonly, the synergist of choice in mosquito control is piperonyl butoxide (PBO).
- Management by multiple methodology. This strategy involves the use of insecticides with different modes of action in mixtures or in rotations. There are economic limitations associated with this approach (e.g., costs and logistics of switching or storing chemicals), and critical variables in addition to the pesticide mode of action that must be taken into consideration (i.e., mode of resistance inheritance, frequency of mutations, population dynamics of the target species, availability of refuges, and migration). Programs should evaluate resistance patterns routinely and the need for rotating insecticides at annual or longer intervals.

Continuing Education

Continuing education for operational vector control workers is required to instill or refresh knowledge related to practical mosquito control. Training focusses on safety, applied technology, and requirements for the regulated certification program mandated by most states. Training should also include information on the identification of mosquito species, their behavior, ecology, and appropriate methods of control.

References

Brogdon WG, McAllister JC. Insecticide resistance and vector control. *Emerg Infect Dis* 1998;4:605–613.

Currier M, et al. Human exposure to mosquito-control pesticides—Mississippi, North Carolina, and Virginia, 2002 and 2003. *MMWR* 2005;54:529–532.

Davis RS, Peterson RK. 2008. Effects of single and multiple applications of mosquito insecticides on nontarget arthropods. *J Am Mosq Control Assoc* 2008;24:270–280.

Duprey Z, et al. Community aerial mosquito control and naled exposure. *J Am Mosq Control Assoc* 2008;24:42–46.

Karpati AM, et al. 2004. Pesticide spraying for West Nile virus control and emergency department asthma visits in New York City, 2000. *Environ Health Perspect* 2004;112:1183–1187.

Lloyd AM, Connelly CR, and Carlson DB (Eds.). Florida Coordinating Council on Mosquito Control. Florida Mosquito Control: The state of the mission as defined by mosquito controllers, regulators, and environmental managers. Vero Beach (FL): University of Florida, Institute of Food and Agricultural Sciences, Florida Medical Entomology Laboratory; 2018.

Macedo, PA, et al. Evaluation of efficacy and human health risk of aerial ultra-low volume applications of pyrethrins and piperonyl butoxide for adult mosquito management in response to West Nile virus activity in Sacramento County, California. *J Am Mosq Control Assoc* 2010;26:57-66.

Peterson RKD, et al. A human-health risk assessment for West Nile virus and insecticides used in mosquito management. *Environ Health Perspect* 2006;114:366-372.

Guidelines for a Phased Response

The objective of a phased response to EEE surveillance data is to implement public health interventions appropriate to the level of risk in a community (Table 2). A surveillance program adequate to monitor EEE virus activity levels associated with human risk must be in place to detect epizootic transmission in advance of human disease outbreaks. Human case reports lag behind human infection events and are poor indicators of current risk levels. Effective public health action depends on interpreting the best available surveillance data and initiating prompt and aggressive intervention when necessary.

Table 2. Recommendations for a Phased Response to EEE Surveillance Data

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 active hospital surveillance for human cases

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Appendix 1. EEE Virus Testing for for Mosquito Pools (Real-Time RT-PCR)

Last Reviewed: December 6, 2022

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Appendix 1: Calculation and Application of a Vector Index (VI) Reflecting the Number of West Nile Virus Infected Mosquitoes in a Population

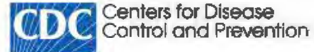
Appendix 2: Interim Guidance for States Conducting Avian Mortality Surveillance for West Nile Virus (WNV) or Highly Pathogenic H5N1 Avian Influenza Virus

About These Guidelines

In the more than 20 years since West Nile virus (WNV) was first detected in New York City, knowledge about WNV epidemiology and transmission ecology has greatly expanded. The objective of this guidance is to consolidate this information and describe how this information can be used to better monitor WNV and mitigate its public health impact. These guidelines are meant for state and local public health officials and mosquito control personnel to aid them in the surveillance and control of WNV.

The fourth version of this guidance was produced in 2013 through a comprehensive review of the published literature related to WNV epidemiology, diagnostics, transmission ecology, environmental surveillance, and vector control. Publications were reviewed for relevance to developing operational surveillance and control programs and selected for inclusion in a draft document by a technical development group of CDC subject matter experts. Numerous stakeholder groups were requested to review the guidance. Comments and additional material provided by National Association of Vector-Borne Disease Control Officials (NAVCO), National Association of City and County Health Officials (NACCHO), Council of State and Territorial Epidemiologists (CSTE), Association of State and Territorial Health Officials (ASTHO), Association of Public Health Laboratories (APHL), and American Mosquito Control Association (AMCA) were incorporated to produce this guidance. We view the recommendations contained in these guidelines as the best that can be derived from the currently available information and will provide updates as new information about WNV epidemiology, ecology, or intervention becomes available. This current version of the guidance has been modified slightly to have them available in an online, electronic format. In general, there are very few changes to the previous guidance though updated epidemiology and references are included.

Last Reviewed: April 12, 2022



Appendix 1: Calculation and Application of a Vector Index (VI) Reflecting the Number of West Nile Virus Infected Mosquitoes in a Population

BACKGROUND. The establishment of West Nile virus (WNV) across North America has been accompanied by expanded efforts to monitor WNV transmission activity in many communities. Surveillance programs use various indicators to demonstrate virus activity. These include detecting evidence of virus in dead birds, dead horses, and mosquitoes; and detection of antibody against WNV in sentinel birds, wild birds, or horses (Reisen & Brault 2007). While all of these surveillance practices can demonstrate the presence of WNV in an area, few provide reliable, quantitative indices that may be useful in predictive surveillance programs. Only indices derived from a known and quantifiable surveillance effort conducted over time in an area will provide information that adequately reflects trends in virus transmission activity that may be related to human risk. Of the practices listed above, surveillance efforts are controlled and quantifiable only in mosquito and sentinel-chicken based programs. In these programs, the number of sentinel chicken flocks/number of chickens, and the number of mosquito traps set per week is known and allows calculation of meaningful infection rates that reflect virus transmission activity.

Premise Behind Developing the Vector Index (VI)

Mosquito-based arbovirus surveillance provides three pieces of information: The variety of species comprising of the mosquito community; density of each species population (in terms of the number collected in each trap unit of a given trap type); and if the specimens are tested for the presence of arboviruses, the incidence of the agent in the mosquito population. Taken individually, each parameter describes one aspect of the vector community that may affect human risk, but the individual elements don't give a comprehensive estimate of the number of potentially infectious vectors seeking hosts at a given time in the surveillance area.

Parameter	Information Provided	Value in Surveillance Program
Mosquito Community Composition	Diversity of species in the area	Documents the presence of competent vector species in the area
Mosquito Population Density	Relative abundance of mosquito species in terms of trapping effort	Quantifies the number of individuals of each mosquito species at a given point in time, particularly important for key vector species
Infection Rate of Virus in Mosquito Population	Proportion of the mosquito population carrying evidence of the disease agent	Quantifies incidence of infected and potentially infectious mosquitoes in the key vector population.

Demonstrates if important bridge vectors are involved

Vector Index

To express the arbovirus transmission risk posed by a vector population adequately, information from all three parameters (vector species presence, vector species density, vector species infection rate) must be considered. The VI combines all three of the parameters quantified through standard mosquito surveillance procedures in a single value (Gujaral et al. 2007, Bolling et al. 2009, Jones et al. 2011, Kwan et al. 2012, Colborn et al. 2013 in press). The VI is simply the estimated average number of infected mosquitoes collected per trap night summed for the key vector species in the area. Summing the VI for the key vector species incorporates the contribution of more than one species and recognizes the fact that WNV transmission may involve one or more primary vectors and several accessory or bridge vectors in an area.

Deriving the VI from routine mosquito surveillance data

The VI is expressed as:

$$\text{Vector Index} = \sum_{i=species} \bar{N}_i \dot{P}_i$$

Where:

$\bar{N}$ = Average Density

(number per trap night for a given species)

$\dot{P}$ = Estimated Infection Rate (proportion of the mosquito population WNV positive)

Calculating the VI in an area where two primary WNV vector species occur:

Step 1: Calculate mosquito density

1	68	21
2	42	63
3	139	49
4	120	31
5	42	12
6	31	57
Total	442	233
Average per Trap Night	74	39
Standard Deviation	41	21

Step 2: Calculate the WNV infection rate for each species (as a proportion)

Pools Tested for Virus

Pools Tested for Virus

Pool Number	Species	Number in pool	Positives
1	*Cx. tarsalis*	50	0
2	*Cx. tarsalis*	50	0
3	*Cx. tarsalis*	50	1
4	*Cx. tarsalis*	50	0
5	*Cx. tarsalis*	50	0
6	*Cx. tarsalis*	50	0
7	*Cx. pipiens*	50	1
8	*Cx. pipiens*	50	0
9	*Cx. pipiens*	50	0
10	*Cx. pipiens*	50	0
11	*Cx. pipiens*	50	0

Cx. tarsalis

Infection Rate	Lower limit	Upper limit	Confidence interval
0.0033	0.0002	0.0169	0.95

Cx. pipiens

Infection Rate	Lower limit	Upper limit	Confidence interval
0.0040	0.0002	0.0206	0.95

Step 3. Calculate individual species VI values, multiplying the average number per trap night by the proportion infected. Calculate combined VI value by summing the individual species VIs.

VI Calculation	*Cx. tarsalis*	*Cx. pipiens*
Avg / trap night	74	39
Proportion infected	0.0033	0.004
VI (individual species)	0.24	0.16

VI (combined)	0.40

References – Vector Index

Bolling BG, Barker CM, Moore CG, Pape WJ, Eisen L. 2009. Seasonal patterns for entomological measures of risk for exposure to *Culex* vectors and West Nile virus in relation to human disease cases in northeastern Colorado. *J Med Entomol.* 46(6):1519-31.

Colborn, J.M., K.A. Smith, J. Townsend, D. Damian, R.S. Nasci, J.P. Mutebi. 2013. West Nile Virus Outbreak in Phoenix, Arizona—2010: Entomological Observations and Epidemiological Correlations. *J Amer Mosq Control Assoc.* In press.

Gujral IB, Zielinski-Gutierrez EC, LeBailly A, Nasci R. 2007. Behavioral risks for West Nile virus disease, northern Colorado, 2003. *Emerg Infect Dis.* 13(3):419-25.

Jones RC, Weaver KN, Smith S, Blanco C, Flores C, Gibbs K, Markowski D, Mutebi JP. 2011. Use of the vector index and geographic information system to prospectively inform West Nile virus interventions. *J Am Mosq Control Assoc* 27:315-319.

Kwan JL, Park BK, Carpenter TE, Ngo V, Civen R, Reisen WK. Comparison of enzootic risk measures for predicting West Nile disease, Los Angeles, California, USA, 2004-2010.2012; *Emerg Infect Dis.*18(8):1298-306.

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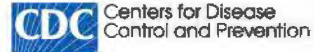
Appendix 2: Interim Guidance for States Conducting Avian Mortality Surveillance for West Nile Virus (WNV) or Highly Pathogenic H5N1 Avian Influenza Virus

Last Reviewed: May 25, 2021



Mosquitoes

Appendix 2: Interim Guidance for States Conducting Avian Mortality Surveillance for West Nile Virus (WNV) or Highly Pathogenic H5N1 Avian Influenza Virus

Appendix 2: Interim Guidance for States Conducting Avian Mortality Surveillance for West Nile Virus (WNV) or Highly Pathogenic H5N1 Avian Influenza Virus

This guidance, which is directed to state health departments, supplements current *West Nile Virus in the United States: Surveillance, Prevention, and Control* and *An Early Detection System for Highly Pathogenic H5N1 Avian Influenza in Wild Migratory Birds: U.S. Interagency Strategic Plan* (https://www.aphis.usda.gov/wildlife_damage/nwdp/pdf/wildbirdstrategicplanpdf.pdf).

Surveillance of dead birds for WNV has proven useful for the early detection of WNV in the United States. In recent months, it has also proven useful for the early detection of highly pathogenic H5N1 avian influenza A (HPAI H5N1, hereafter referred to as H5N1 virus) in Europe. Given the potential for H5N1 to infect wild birds in North America in the future, the following interim guidance is offered to support the efforts of states conducting avian mortality surveillance.

General Considerations for States Conducting Avian Mortality Surveillance

- If different agencies within a state are separately responsible for conducting surveillance for WNV or H5N1 among wild birds, the sharing of resources, including dead birds submitted for testing, may increase the efficiency of both systems.
- Any dead bird might be infected with any one of a number of zoonotic diseases currently present in the United States, such as WNV. However, in countries where H5N1 has been found in captive and wild birds, it frequently has resulted in multiple deaths within and across species, and if H5N1 enters the US, it is likely to result in the death of wild birds. If wild birds in the United States are exposed to the virus, both single and groups of dead birds should be considered potentially infected.

Avian mortality due to the introduction of H5N1 could occur at any time of the year, whereas WNV is more often detected when mosquitoes are active.

- To date, no human infections of WNV have been confirmed due to contact with live or dead wild birds in outdoor settings.
- Most human H5N1 cases overseas have been associated with close contact with infected poultry or their environment; however, a very small number of cases appear to be related to the handling of infected wild birds or their feathers or feces without the use of proper personal protective equipment (PPE). There is no evidence of H5N1 transmission to humans from exposure to H5N1 virus-contaminated water during swimming; however, this may be theoretically possible. (https://www.who.int/news-room/fact-sheets/detail/influenza-(avian-and-other-zoonotic))
- Although handling infected birds is unlikely to lead to infection, persons who develop an influenza-like illness after handling sick or dead birds should seek medical attention. Their health care provider should report the incident to public health agencies if clinical symptoms or laboratory test results indicate possible H5N1 or WNV infection.

Infection Control and Health and Safety Precautions

These guidelines are intended for any person handling dead birds. The risk of infection with WNV from such contact is small. The risk of infection with H5N1 from handling dead birds is difficult to quantify and is likely to vary with each situation. Risk is related to the nature of the work environment, the number of birds to be collected, and the potential for aerosolization of bird feces, body fluids, or other tissues. **The most important factor that will influence the degree of infection risk from handling wild birds is whether H5N1 has been reported in the area.** Local public health officials can be consulted to help in selecting the most appropriate PPE for the situation.

General Precautions for Collection of Single Dead Birds (These precautions are applicable to employees as well as the general public)

When collecting dead birds, the risk of infection from WNV, H5N1, or any other pathogen may be eliminated by avoiding contamination of mucous membranes, eyes, and skin by material from the birds. This can be accomplished by eliminating any direct contact with dead birds via use of the following safety precautions:

- When picking up any dead bird, wear disposable impermeable gloves and place it directly into a plastic bag. Gloves should be changed if torn or otherwise damaged. If gloves are not available, use an inverted double-plastic bag technique for picking up carcasses or use a shovel to scoop the carcass into a plastic bag.

In situations in which the bird carcass is in a wet environment or in other situations in which splashing or aerosolization of viral particles is likely to occur during disposal, safety goggles or glasses and a surgical mask may be worn to protect mucous membranes against splashed droplets or particles.

Bird carcasses should be double bagged and placed in a trash receptacle that is secured from access by children and animals. If the carcass will be submitted for testing, hold it a cool location until it pickup or delivery to authorities. Carcasses should not be held in close contact with food (e.g., not in a household refrigerator or picnic cooler).

After handling any dead bird, avoid touching the face with gloved or unwashed hands.

Any PPE that was used (e.g. gloves, safety glasses, mask) should be discarded or disinfected* when done, and hands should then be washed with soap and water (or use an alcohol-based hand gel when soap and water are not available). http://www.cdc.gov/cleanhands/

- If possible, before disposing of the bird, members of the public may wish to consult with their local animal control, health, wildlife or agricultural agency or other such entity to inquire whether dead bird reports are being tallied and if the dead bird in question might be a candidate for WNV or H5N1 testing.

Additional Precautions for Personnel Tasked with Collecting Dead Birds in Higher-Risk Settings (e.g., when collecting large numbers or in confined indoor spaces, particularly once H5N1 has been confirmed in an area)

- Minimize any work activities that generate airborne particles. For example, during the cleanup phase of the bird removal, avoid washing surfaces with pressurized water or cleaner (i.e., pressure washing), which could theoretically aerosolize H5N1 viral particles that could then be inhaled. If aerosolization is unavoidable, the use of a filtering face-piece respirator (e.g., N95) would be prudent, particularly while handling large quantities of dead birds repeatedly as part of regular work requirements.

If using safety glasses, a mask, or a respirator, do not remove until after gloves have been removed and hands have been washed with soap and water (or use an alcohol-based hand gel when soap and water are not available). After PPE has been removed, hands should immediately be cleaned again (http://www.cdc.gov/cleanhands/) Personal protective equipment worn (e.g., gloves, mask, or clothing) should be disinfected* or discarded.

Discuss appropriate biosafety practices and PPE use with your employer.

*Recommendations for PPE Disinfection

For machine-washable, reusable PPE: Disinfect PPE in a washing machine with detergent in a normal wash cycle. Adding bleach will increase the speed of viral inactivation as will hot water but detergent alone in cold water will be effective. Follow manufacturer recommendations for drying the PPE. Non machine-washable, reusable PPE should be cleaned following the manufacturer's recommendations for cleaning.

Laboratory Biosafety Recommendations

Laboratory handling of routine diagnostic specimens of avian carcasses requires a minimum of BSL-2 laboratory safety precautions. However, if either WNV or H5N1 infection of the specimens is suspected on the basis of previous surveillance findings, at a minimum BSL-3 precautions are advisable. Consult your institutional biosafety officer for specific recommendations. Biosafety levels are described at www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4s3.htm.

Additional Information Sources

Interim Guidance for Protection of Persons Involved in U.S. Avian Influenza Outbreak Disease Control and Eradication Activities (http://www.cdc.gov/flu/avian/professional/protect-guid.htm)

Interim Guidelines for the Protection of Persons Handling Wild Birds with Reference to Highly Pathogenic Avian Influenza H5N1 (https://www.usgs.gov/media/files/interim-guidelines-protection-persons-handling-wild-birds)

Avian Influenza: Protecting Workers at Risk (https://www.osha.gov/dts/shib/shib121304.html)

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Appendix 1: Calculation and Application of a Vector Index (VI) Reflecting the Number of West Nile Virus Infected Mosquitoes in a Population

› **Appendix 2: Interim Guidance for States Conducting Avian Mortality Surveillance for West Nile Virus (WNV) or Highly Pathogenic H5N1 Avian Influenza Virus**

Last Reviewed: April 28, 2022

Mosquitoes

ArboNET

ArboNET

ArboNET, the national arboviral surveillance system, was developed by CDC and state health departments in 2000 in response to the emergence of WNV in 1999. Since its development, ArboNET has expanded to include many other arboviruses of public health importance. ArboNET is an electronic surveillance system administered by CDC's Division of Vector-Borne Diseases. Human arboviral disease data are reported from all states, territories, and freely associated states. In addition to human disease cases, ArboNET maintains data on arboviral infections among human viremic blood donors, non-human mammals, sentinel animals, dead birds, and mosquitoes.

Data Collected. Variables collected for human disease cases include patient age, sex, race, and county and state of residence; date of illness onset; case status (i.e., confirmed, probable, suspected, or not a case); clinical syndrome (e.g., encephalitis, meningitis, or uncomplicated fever); whether illness resulted in hospitalization; and whether the illness was fatal. Cases reported as encephalitis (including meningoencephalitis), meningitis, or acute flaccid paralysis are collectively referred to as neuroinvasive disease; others are considered non-neuroinvasive disease. Acute flaccid paralysis can occur with or without encephalitis or meningitis. Information regarding potential non-mosquito-borne transmission (e.g., blood transfusion or organ transplant recipient, breast-fed infant, or laboratory worker) and recent donation of blood or solid organs should be reported if applicable. Clinical symptoms and diagnostic testing data can also be reported.

Blood donors identified as presumptively viremic by nucleic acid amplification test (NAAT) screening of the donation by a blood collection agency are also reported to ArboNET. Case definitions have been developed for the purposes of national surveillance. The date of blood donation is reported in addition to the variables routinely reported for disease cases.

Arboviral disease in non-human mammals (primarily horses) and infections in trapped mosquitoes, dead birds, and sentinel animals (primarily chickens) are also reported to ArboNET. Variables collected for non-human infections include species, state and county, and date of specimen collection or symptom onset. The total number of mosquitoes or birds tested weekly can also be reported by county and species.

Detailed descriptions of all variables collected by ArboNET and instructions for reporting are included in the ArboNET User Guide, which can be requested from DVBD by phone (970-261-6400) or email (dvbid2@cdc.gov).

Data Transmission. Jurisdictions can transmit data to ArboNET using one or more of four methods supported by DVBD: 1) jurisdictions that have a commercially- or state-developed electronic surveillance system can upload records from their system using an Extensible Markup Language (XML) message; 2) jurisdictions can upload records from a Microsoft® Access database provided by CDC DVBD using an XML message; 3) jurisdictions may enter records manually using a CDC website (https://csams.cdc.gov/arbonet); or 4) jurisdictions can report cases using an HL-7 message via the CDC National Electronic Disease Surveillance System (NEDSS) and DVBD will download records directly from NEDSS to ArboNET. ArboNET data are maintained in a Microsoft® Structured Query Language (SQL) Server® database inside CDC's firewall. Users can access data via a password-protected website but are limited to viewing data only from their own jurisdiction. The ArboNET website and database are maintained by CDC information technology staff and are backed up nightly.

Dissemination of ArboNET Data. CDC epidemiologists periodically review and analyze ArboNET surveillance data and disseminate results to stakeholders via direct communication, briefs in *Morbidity and Mortality Weekly Reports* and Epi-X, comprehensive annual summary reports, and DVBD's website. CDC also produces maps of domestic and exotic arboviral

activity, which are then posted on a website (https://wwwn.cdc.gov/arbonet/maps/ADB_Diseases_Map/index.html). Surveillance reports are typically updated biweekly during the transmission season and monthly during the off-season. A final report is usually released in the spring of the following year. CDC provides limited-use ArboNET data sets to the general public by formal request. Data release guidelines have been updated to be consistent with those developed by CDC and the Council of State and Territorial Epidemiologists (CSTE).

Limitations of ArboNET Data. Human surveillance for arboviral disease is largely passive, and relies on the receipt of information from physicians, laboratories, and other reporting sources by state health departments. For viruses that can cause neuroinvasive disease, neuroinvasive disease cases are likely to be consistently reported because of the substantial morbidity associated with this clinical syndrome. In comparison, non-neuroinvasive disease cases are inconsistently reported because of a less severe spectrum of illness, geographic differences in disease awareness and healthcare seeking behavior, and variable capacity for laboratory testing. Surveillance data for fever cases associated with neuroinvasive arboviruses should be interpreted with caution and generally should not be used to make comparisons between geographic areas or over time. Accordingly, ratios of reported neuroinvasive disease cases to non-neuroinvasive disease cases should not be interpreted as a measure of virulence in an area.

ArboNET does not routinely collect information regarding clinical signs and symptoms or diagnostic laboratory test results. Therefore, misclassification of the various syndromes caused by arboviruses cannot be detected. In addition, ArboNET does not routinely collect information regarding the specific laboratory methods used to confirm each case. Although serologic assays are relatively specific, false-positive results and cross-reactions occur between related viruses (e.g., flavivirus, such as West Nile, St. Louis encephalitis, and dengue viruses, or California serogroup viruses, such as La Crosse and Jamestown Canyon viruses). Positive IgM results should be confirmed by additional tests, especially plaque-reduction neutralization. However, such confirmatory testing often is not performed. While the electronic mechanisms for data transmission allow for rapid case reporting, the inclusion of both clinical and laboratory criteria in the surveillance case definition creates delays between the occurrence of cases and their reporting. Provisional data are disseminated to allow for monitoring of regional and national epidemiology during the arboviral transmission season. However, these reports generally lag several weeks behind the occurrence of the cases comprising them, and the data may change substantially before they are finalized. For this reason, provisional data from the current transmission season should not be combined with or compared to provisional or final data from previous years.

The collection and reporting of non-human surveillance data are highly variable among states (and even between regions within states) and changes from year to year. Because of this variability, non-human surveillance data should not be used to compare arboviral activity between geographic areas or over time.

For more information about ArboNET, please contact the Division of Vector-Borne Diseases by phone: 970-261-6400 or email: dvbid2@cdc.gov.

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Last Reviewed: April 26, 2022

Environmental Surveillance

Vector-based Surveillance

Vector surveillance is an integral component of an Integrated Vector Management (IVM) program and is the primary tool for quantifying virus transmission and human risk. The principal functions of a mosquito-based surveillance program are to:

- Collect data on mosquito population abundance and virus infection rates in those populations.
- Provide indicators of the threat of human infection and identify geographic areas of high-risk.
- Support decisions regarding the need for and timing of intervention activities (e.g. enhanced vector surveillance and control, use of new technologies and public education programs).
- Monitor the effectiveness of vector control methods, including susceptibility of target mosquitoes to control methods used.

Mosquito-based arboviral monitoring complements disease surveillance programs by contributing fast results and data for action. Programs maintaining in-house laboratories can process mosquito samples daily, giving results within a few days. Data on vector species community composition, relative abundance, and infection rates allow programs to rapidly compute infection indices, assess risk and respond. Maintaining mosquito surveillance over the long-term provides a baseline of historical data to evaluate risk and guide mosquito control operations. However, the utility of mosquito-based surveillance depends both on the type and quality of data collected (e.g., number and type of traps, timing and frequency of sampling, testing procedures) and consistent effort across transmission seasons to link surveillance indices with human risk.

There are three main categories of vector surveillance: larval, adult, and transmission activity. Together, this information is used to determine where and when control efforts should be implemented. Larval surveillance involves sampling a wide range of aquatic habitats to identify the sources of vector mosquitoes and evaluating larval control measures applied. For adult mosquitoes, regular (e.g. monthly, weekly) sampling is done at fixed sites throughout the community that are representative of the habitat types present in the area. Adult mosquitoes are collected using a variety of trapping techniques, including traps for host-seeking, resting, or gravid (carrying eggs) mosquitoes seeking a place to lay eggs (oviposition site).

Specimen Collection and Traps

Light traps collect a wide range of mosquito species (McCardle et al. 2004), providing information about both primary and secondary vectors and a better understanding of the species composition in an area. The three major WNV and St. Louis encephalitis vectors (*Cx. pipiens*, *Cx. quinquefasciatus*, and *Cx. tarsalis*) can be collected in light traps. However, light traps may collect fewer *Cx. pipiens* or *Cx. quinquefasciatus* resulting in small sample sizes and less accurate estimates of WNV infection rates.

CDC miniature light traps (Sudia and Chamberlain 1962) are lightweight and use batteries to provide power to a light source and fan motor. CO_2 (usually dry ice) is frequently used as an additional attractant. Light traps have several considerations:

- Collections may consist largely of unfed, nulliparous individuals, which greatly reduces the likelihood of detecting WNV and other arboviruses.
- Not all mosquito species are attracted to light traps and the numbers captured may not reflect the population size of a particular species.
- For day-time active mosquitoes other trapping methods should be considered (CDC 2016).

Gravid traps can be useful for sampling *Cx. pipiens* and *Cx. quinquefasciatus*, particularly in urban areas (Andreadis and Armstrong 2007, Reisen et al. 1999). Because gravid females have previously taken a blood meal, this increases the likelihood of capturing infected mosquitoes and detecting virus. Gravid traps can be baited with attractants such as fresh or dry grass clipping infusions, rabbit chow infusions, cow manure, fish oil, or other materials that mimic the stagnant water in habitats where these species lay eggs. These vary in attractiveness depending on the type of infusion and its preparation (Burkett et al. 2004, Lampman et al. 1996). Gravid traps mainly capture mosquitoes in the *Cx. pipiens* complex, and therefore provide limited information on overall species composition within a region (Reiter et al. 1986).

Collecting resting mosquitoes provides a good representation of vector population structure and underlying WNV infection rates, since unfed, gravid, and blood-fed females (as well as males) may be collected. Resting mosquitoes can be collected using suction traps such as the CDC resting trap (Panella et al. 2011), and by using handheld or backpack mechanical aspirators (Nasci 1981) to remove mosquitoes from natural resting harborages or artificial resting structures (e.g., wooden resting boxes, red boxes, fiber pots, and other similar containers). Because of the wide variety of resting sites and the low density of resting mosquitoes in most locations, sampling resting populations is labor intensive and sufficient sample sizes are often difficult to obtain.

Host-baited traps, often employing chickens or pigeons as bait, can collect large numbers of mosquitoes of interest. However, these methods require live animals and adherence to animal use requirements and permitting. The bait species and variations in individual host attractiveness can impact trap performance. These traps target host-seeking mosquitoes and therefore collect mainly unfed, nulliparous individuals.

Human landing collections may expose collectors to infected mosquitoes and are not recommended as a sampling procedure in areas where WNV transmission is occurring.

Specimen Handling and Processing

Since mosquito-based surveillance relies on identifying virus in the collected mosquitoes through detection of viral proteins, viral RNA, or live virus (see Laboratory Diagnosis and Testing section), specimens should be handled in a way that minimizes exposure to conditions (e.g., heat, successive freeze-thaw cycles) that would degrade the virus. Optimally, a cold chain should be maintained from the time mosquitoes are removed from the traps to the time they are delivered to the processing laboratory. Mosquitoes can be transported from the field in a cooler with cold packs or on dry ice, and then placed on a chill-table, if available, during sorting identification, and pooling. Usually only female mosquitoes are tested in routine arboviral surveillance programs. If virus screening is not done immediately after mosquito identification and pooling, the pooled samples should be stored frozen (e.g. -70°C) or at temperatures below freezing for short-term storage. Lack of a cold chain does not appear to reduce the ability to detect viral RNA by reverse transcription polymerase chain reaction (RT-PCR) for WNV (Turell et al. 2002).

Vector-based Surveillance Indicators

Data derived from mosquito surveillance include estimates of mosquito species abundance and infection rate in those mosquito populations. The indices derived from those data vary in information content, ability to be compared over time and space, and association with transmission levels and levels of human risk. Five indicators that have commonly been used: vector abundance, number of positive pools, percent of pools positive, infection rate, and vector index. (Table)

Vector abundance provides a measure of the relative number of mosquitoes in an area during a particular sampling period. It is the total number of mosquitoes of a particular species collected, divided by the number of trapping nights during a specified sampling period, and is expressed as the number/trap night. Risk assessments often consider mosquito abundance because high mosquito densities can be associated with arboviral disease outbreaks (Olson et al. 1979, Eldridge 2004). For example, during a WNV outbreak in Maricopa County, Ariz., 2010, *Cx. quinquefasciatus* densities were higher in outbreak compared to non-outbreak areas (Godsey et al. 2012, Colborn et al. 2013). However, high mosquito abundance can occur in the absence of virus and outbreaks can occur when abundance is low, but the vector

infection rate is high. Vector abundance measures are also used for planning IVM and monitoring the outcomes of mosquito control. Number of traps, their distribution, and the timing of sample collection should be sufficient to obtain spatially and temporally representative data.

Number of positive pools is the total of the number of arbovirus positive mosquito pools detected in a given surveillance location and period. These may be a tally of the total positive pools separated by species or for all species tested. This indicator provides evidence of WNV activity but is not recommended as a stand-alone indicator. Instead, data can be used to produce more informative indices (i.e., Infection Rate and Vector Index).

Percent of pools positive is calculated by the number of positive pools divided by the total number of pools tested, as a percentage. It provides a rough estimate of the rate of infection and can be used to compare activity over time and place. However, the comparative value is limited unless the number of pools tested is large and the number of mosquitoes per pool remains constant. As with the number of positive pools index, these data can be used for the (more informative) Infection Rate and Vector Index.

The **Infection Rate** in a vector population estimates the prevalence of infected mosquitoes in the population and is a good indicator of human risk. It provides a useful, quantitative basis for comparison, allowing evaluation of changes in infection rate over time and space. Infection rate indices have been used successfully to link infection rates with human risk (Bell et al. 2005). Variable pool numbers and pool sizes can be used, while retaining comparability, but larger sample sizes improve accuracy. Two methods are commonly used to calculate infection rate:

- **Minimum infection rate (MIR)** for a given mosquito species is the number of positive pools divided by the total number of mosquitoes tested. MIR assumes that infection rates are low and that only one mosquito is positive in a positive pool. MIR is usually expressed as the number infected/1000 tested. It can also be expressed as a proportion or percent positive.
- **Maximum likelihood estimate (MLE)** is the preferred method, particularly during outbreaks. MLE does not assume only one positive mosquito per positive pool and provides a more accurate estimate when infection rates are high (Gu et al. 2008). The MLE and MIR are similar when infection rates are low. The MLE requires more complex calculations than the MIR; however, a Microsoft Excel® Add-In to compute infection rates from pooled data is available (https://www.cdc.gov/westnile/resourcepages/mosqSurvSoft.html).

The **Vector Index (VI)** estimates the abundance of infected mosquitoes in an area and incorporates into a single index information on presence, relative abundance, and infection rates of individual species (Gujral et al. 2007, Bolling et al. 2009, Jones et al. 2011). The VI is calculated by multiplying the average number of mosquitoes collected per trap night by the proportion infected. VI is expressed as the average number of infected mosquitoes collected per trap night in the area during the sampling period. In areas with multiple vector species, a VI is calculated for each species. Individual VIs are summed to give a combined estimate of infected vector abundance.

Increases in VI reflect increased risk of human disease and are more reliable prediction measures than vector abundance or infection rate alone (Bolling et al. 2009, Jones et al. 2011, Kwan et al. 2012, Colborn et al. 2013). As with other surveillance indicators, the accuracy of the VI depends on the number of trap nights used to estimate abundance and the number of specimens tested to estimate infection rate. Instructions for calculating the VI in a system with multiple vector species are in Appendix 1.

Use of Vector-based Surveillance Indicators

Mosquito-based surveillance indicators have two important roles in arboviral surveillance and response programs. First, they can provide quantifiable thresholds for proactive vector control efforts. By identifying thresholds for vector abundance and infection rate that are below levels associated with disease outbreaks, IVM programs can institute proactive measures to maintain mosquito populations at levels below which virus amplification can occur. Second, if thresholds related to outbreak levels of transmission can be identified, surveillance can help determine when proactive measures were insufficient to dampen virus amplification and more aggressive measures, such as wide-scale aerial application of mosquito adulticides and expanded public messaging, are needed to stop an outbreak.

Summary of Mosquito-Based Surveillance Indicators

Index	Description	Equation

Index	Description	Equation
Vector Abundance	Number of mosquitoes of a particular vector species captured per trap per night	Number of a particular mosquito species captured in a night/Number of traps set up that night
Number of Positive Mosquito Pools	Number of positive mosquito pools detected in a given period of time	Simple count of positive mosquito pools
Percentage of Positive Mosquito Pools	Proportion of positive mosquito pools	Number of positive mosquito pools/Total number of pools tested X 100
Infection Rate	An estimate of the number of mosquitoes infected per 1000 tested	Minimum Infection Rate (MIR) = Number of positive pools/Total number of mosquitoes tested Maximum likelihood estimate (MLE), use links in the footnote.
Vector Index	An estimate of the abundance of infected mosquitoes in an area	$\overline{N}$ = Number of mosquitoes per trap night for a given species $\hat{P}$ = Estimated Infection Rate $\text{Vector Index} = \sum_{i=species} \overline{N}_i \hat{P}_i$

For MLE computations use the mosquito surveillance software at https://www.cdc.gov/westnile/resourcepages/mosqSurvSoft.html

References

Andreadis TG, Armstrong PM. 2007. A two-year evaluation of elevated canopy trapping for *Culex* mosquitoes and West Nile virus in an operational surveillance program in the Northeastern United States. *J Am Mosq Control Assoc*. 23(2):137-148.

Bell JA, Mickelson NJ, Vaughan JA. 2005. West Nile virus in host-seeking mosquitoes within a residential neighborhood in Grand Forks. *North Dakota Vector-Borne Zoonotic Dis*. 5:373

Bolling BG, Barker CM, Moore CG, Pape WJ, Eisen L. 2009. Modeling/GIS, risk assessment, economic impact: Seasonal patterns for entomological measures of risk for exposure to *Culex* vectors and West Nile virus in relation to human disease cases in Northeastern Colorado. *J Med Entomol*. 46:1519-1531.

Burkett DA, Kelly R, Porter CH, Wirtz RA. 2004. Commercial mosquito trap and gravid trap oviposition media evaluation, Atlanta, Georgia. *J Am Mosq Control Assoc*. 20(3): 223-228.

CDC. 2016. Surveillance and Control of *Aedes aegypti* and *Aedes albopictus* in the United States. https://www.cdc.gov/mosquitoes/mosquito-control/professionals/index.html (accessed 08/31/2021)

Colborn, J.M., K.A. Smith, J. Townsend, D. Damian, R.S. Nasci, J.P. Mutebi. 2013. West Nile Virus Outbreak in Phoenix, Arizona—2010: Entomological Observations and Epidemiological Correlations. *J Amer Mosq Control Assoc*. 29(2):123-32.

Eldridge BF. 2004. Surveillance for arthropodborne diseases. In Eldridge and Edman eds. Medical Entomology, Kluwer Academic Press, Dordrecht, The Netherlands, pp 645

Godsey MS Jr., Burkhalter K, Young G, Delorey M, Smith K, Townsend J, Levy C, Mutebi JP. 2012. Entomologic investigations during an outbreak of West Nile virus disease in Maricopa County, Arizona, 2010. *Am J Trop Med Hyg*. 87(6):1125-1131.

Gu W, Unnasch TR, Katholi CR, Lampman R, Novak RJ. 2008. Fundamental issues in mosquito surveillance for arboviral transmission. *Trans R Soc Trop Med Hyg.* 102: 817-822.

Gujral IB, Zielinski-Gutierrez EC, LeBailly A, Nasci R. 2007. Behavioral risks for West Nile virus disease, northern Colorado, 2003. *Emerg Infect Dis.* 13(3):419-25.

Jones RC, Weaver KN, Smith S, Blanco C, Flores C, Gibbs K, Markowski D, Mutebi JP. 2011. Use of the vector index and geographic information system to prospectively inform West Nile virus interventions. *J Am Mosq Control Assoc.* 27:315-319.

Kwan JL, Park BK, Carpenter TE, Ngo V, Civen R, Reisen WK. 2012. Comparison of enzootic risk measures for predicting West Nile disease, Los Angeles, California, USA, 2004-2010. *Emerg Infect Dis.* 18(8):1298-306.

Lampman RL, Novak RJ. 1996. Oviposition preferences of *Culex pipiens* and *Culex restuans* for infusion-baited traps. *J Am Mosq Control Assoc.* 12(1):23-32.

McCardle PW, Webb RE, Norden BB, Aldrich JR., 2004. Evaluation of five trapping systems for the surveillance of gravid mosquitoes in Prince Georges County, Maryland. *J Am Mosq Control Assoc.* 20(3):254-260.

Nasci RS. 1981. A lightweight battery-powered aspirator for collecting resting mosquitoes in the field. *Mosq News.* 41: 808-811.

Olson JG, Reeves WC, Emmons RW, Milby MM. 1979. Correlation of Culex tarsalis population indices with the incidence of St. Louis encephalitis and western equine encephalomyelitis in California. *Am J Trop Med Hyg.* 28: 335-343.

Panella NA, Crockett RJ, Biggerstaff BJ, Komar N. 2011. The Centers for Disease Control and Prevention resting trap: a novel device for collecting resting mosquitoes. *J Am Mosq Control Assoc.* 27(3):323-325.

Reisen WK, Boyce K, Cummings RC, Delgado O, Gutierrez A, Meyer RP, Scott TW. 1999. Comparative effectiveness of three adult mosquito sampling methods in habitats representative of four different biomes of California. *J Med Entomol.* 36(1):23-29.

Reiter P, Jakob WL, Francy DB, Mullenix JB. 1986. Evaluation of the CDC gravid trap for the surveillance of St. Louis encephalitis vectors in Memphis, Tennessee. *J Am Mosq Control Assoc.* 2(2):209-211.

Sudia WD, Chamberlain RW. 1962. Battery-operated light trap an improved model. *Mosq News*, 22: 126-129.

Turell MJ, Spring AR, Miller MK, Cannon CE. 2002. Effect of holding conditions on the detection of West Nile viral RNA by reverse transcriptase-polymerase chain reaction from mosquito (Diptera: Culicidae) pools. *J Med Entomol.* 39(1):1-3.

Animal-based Surveillance

Bird-based Surveillance

WNV amplifies in nature by replicating to high levels in a variety of bird species (326 affected species reported to ArboNET through 2016; CDC 2016), which then transmit the virus to mosquitoes during several days of sustained high-level viremia. In addition to infection from mosquito bites, some birds are infected by consuming infected prey (insects, small mammals, other birds) or in rare cases, from direct contact with other infected birds. A hallmark of the North American strain of WNV is its propensity to kill many birds it infects. Corvids (species of the family *Corvidae*, including crows, ravens, magpies, and jays) and other select species are particularly susceptible (Komar 2003). Avian morbidity/mortality surveillance and monitoring infections in wild or captive birds are strategies used to determine WNV activity and can provide a quantitative index of risk for human infection.

Avian Morbidity/Mortality Surveillance

Dead bird reporting systems collect broad information about the temporal and spatial patterns of bird deaths in an area and provide insight into WNV activity. Public participation is essential and must be encouraged through an effective public education and outreach program. A system for carcass reporting should be established including a database to record and analyze dead bird sightings with the following suggested data: caller identification and call-back number, date observed, location geocoded to the highest feasible resolution, species, and condition. A subset of the reported bird deaths can be investigated to confirm WNV activity. Birds in good condition (not scavenged and without obvious decomposition or maggot infestation) may be sampled or retrieved for laboratory testing (see Avian morbidity/mortality testing). Dead bird reporting systems provide a wide surveillance net extending to any area where a person is present to observe a dead bird. These systems have been used with success to estimate risk of human infection (Eidson et al. 2001a, Mostashari et al. 2003, Carney et al. 2011).

There are several limitations to dead bird surveillance systems. Maintaining public interest and willingness to participate is essential to these programs but is difficult to maintain. The surveillance is passive and qualitative and can only be used to assess risk of infection to people in areas where sufficient data are collected to populate risk models such as DYCAST (Carney et al. 2011) and SaTScan (Mostashari et al. 2003). Over time, bird populations can become resistant to morbidity and mortality (Reed et al. 2009), compromising the utility of this surveillance for WNV. Other causes of bird mortality could cause a false alarm for WNV activity, although this might also alert the public health and wildlife disease communities to other pathogens or health threats.

In programs where the objective of avian morbidity/mortality testing is early detection of WNV activity and not a quantitative index of human risk, testing dead birds should be initiated when local adult mosquito activity begins in the spring, and continue as long as local WNV activity is undetected in the area. Once WNV is detected in dead birds, or if vector prevention and control actions have been initiated, continued detection of WNV in carcasses in that area does not provide additional information about WNV activity and is not necessary or cost-effective. However, the number of WNV-infected dead birds can contribute to an effective human risk index (Kwan et al. 2012a).

Contact with WNV-infected carcasses presents a potential health hazard to handlers (Fonseca et al. 2005). Appropriate biosafety precautions should be taken when handling carcasses in the field and in the laboratory. More detailed guidelines for sampling avian carcasses are available in Appendix 2.

To maximize sensitivity of this surveillance system, a variety of bird species should be tested, but corvids should be emphasized if they are present (Nemeth et al. 2007a). In dead corvids and other birds, bloody pulp from immature feathers, and tissues collected at necropsy such as brain, heart, kidney, or skin harbor very high viral loads, and any of these specimen types is sufficient for sensitive detection of WNV (Panella et al. 2001, Komar et al. 2002, Docherty et al. 2004, Nemeth et al. 2009, Johnson et al. 2010). Oral swabs and breast feathers are easy specimens to collect in the field, avoid the need to transfer dead birds to the laboratory, do not require a cold chain, and are effective for detecting WNV in dead corvids (Komar et al. 2002, Nemeth et al. 2009). They are less sensitive for WNV detection in non-corvids; however, the reduced sensitivity of testing non-corvids using these tissue types can be offset by sampling more carcasses. The number of bird specimens tested will be dependent upon resources and whether WNV-infected birds have already been found in the area; triage of specimens by species or by geographic location may be appropriate in some jurisdictions.

Several studies have demonstrated the effectiveness of avian mortality testing for early detection of WNV activity (Eidson et al. 2001b, Julian et al. 2002, Guptill et al. 2003, Nemeth et al. 2007b, Patnaik et al. 2007, Kwan et al. 2012a). Wildlife rehabilitation clinics can be a good source of specimens derived from carcasses (Nemeth et al. 2007b). Collecting samples from living birds that are showing signs of illness requires the assistance of a veterinarian or wildlife technician. Dead crows and raptors alarm the public and carcasses are easily spotted. However, in regions with few or no crows, carcasses may be less obvious. Eye aspirates have been shown to be a sensitive and fast sampling protocol for WNV detection in corvid carcasses brought to the laboratory for testing (Lim et al 2009).

Live Bird Serology

The use of living birds as sentinels for monitoring WNV transmission requires serially blood-sampling a statistically valid number of avian hosts. Captive chickens, frequently referred to as sentinel chickens, (though other species have been used) provide the most convenient source of blood for this purpose. Blood may be collected from a wing vein, the jugular vein, or on Nobuto® strips by pricking the chicken's comb with a lancet. There is no standard protocol for implementing a sentinel chicken program. It can be tailored to the specific circumstances of each surveillance jurisdiction, though sentinel

chicken systems generally employ flocks of 6-10 birds at each site and bleed each bird weekly or every other week throughout the WNV transmission season. Sentinel chicken-based WNV surveillance systems can provide evidence of WNV transmission several weeks in advance of human cases (Healy et al. 2012).

While serially sampling free-ranging bird species is very labor intensive, it can provide information about seroconversion in amplifier hosts, similar to the data provided by sentinel chickens. Quantifying seroprevalence in free-ranging birds may provide additional information that benefits surveillance programs (Komar 2001). For example, a serosurvey of the local resident bird population (in particular, juvenile birds) following the arbovirus transmission season may help determine which local species may be important amplifiers of WNV in the surveillance area. This in turn could be used to map areas of greatest risk in relation to the populations of amplifier hosts. Furthermore, a serosurvey of adult birds just prior to arbovirus transmission season can detect pre-existing levels of antibody in the bird population. High levels would suggest less opportunity for WNV amplification because many adult bird species transfer maternal antibodies to their offspring, which can delay or inhibit WNV amplification among the population of juvenile birds that emerges each summer. In Los Angeles, California, serosurveys of local amplifier hosts during winter determined that subsequent outbreaks occurred only after seroprevalence dipped below 10% in these birds (Kwan et al. 2012b).

There are several advantages of sentinel chicken and other live-bird serology surveillance systems. Sentinel chickens are captive, so a seroconversion event indicates local transmission and presence of infected mosquitoes in the area. Chickens do not develop clinical disease, nor do they develop viremias sufficient to infect mosquitoes (Langevin et al. 2001). Chickens are preferred blood-feeding hosts of *Cx. pipiens* and *Cx. quinquefasciatus*, which are important urban vectors of WNV. Chickens can be used to monitor seroconversions of multiple arboviruses of public health importance (i.e., WNV, SLE, WEE, and EEE viruses) simultaneously. However, there are also a number of important limitations related to these systems. Determination that a chicken has seroconverted occurs typically 3-4 weeks after the transmission event has occurred and reporting of a positive chicken may not precede the first local case of human disease caused by WNV (Patnaik et al. 2007, Kwan et al. 2010, Unlu et al. 2009). Use of sentinel birds requires institutional animal use and care protocols, and other authorization permits. Linking patterns in sentinel chicken seroconversion with human risk requires multiple years of data.

Horses and Other Vertebrates

Horses are susceptible to encephalitis due to WNV infection; thus, equine cases of WNV-induced encephalitis may serve a sentinel function in the absence of other environmental surveillance programs. Equine health is an important economic issue, so severe disease in horses comes to the attention of the veterinary community. Use of horses as sentinels for active WNV surveillance is theoretically possible, but practically infeasible. Widespread use of equine WNV vaccines decreases the incidence of equine WNV disease, and survivors of natural infections are protected from disease, reducing the usefulness of equines as sentinels. Veterinarians, veterinary service societies/agencies, and state agriculture departments are essential partners in any surveillance activities involving WNV infections in horses. Equine disease due to WNV is rare in tropical ecosystems. However, WNV frequently infects horses in the tropics. Detection of seroconversions in horses has been suggested as a sentinel system to detect risk of WNV transmission to people in Puerto Rico and other tropical locations (Phoutrides et al. 2011, Mattar et al. 2011).

Small numbers of other mammal species have been affected by WNV. Dead squirrels are tested for WNV along with dead birds in some jurisdictions. Among domestic mammals, the most important has been the camelids, such as llamas and alpacas. As with horses, these come to the attention of veterinarians and any veterinary case of disease due to WNV may be used for passive surveillance. Dogs and cats become infected with WNV. Active surveillance of WNV in dogs has been shown to predict human infection with WNV (Resnick et al. 2008). WNV disease in dogs is rare and vaccination of dogs has not been recommended or practiced. Maintaining a large number of seronegative dogs for use as sentinels would be cumbersome, but juvenile stray dogs could be used for this purpose in areas where other surveillance methods are not available. Stray dog removal programs could provide a source of samples at low cost. WNV infects cats but cats have not been evaluated as surveillance sentinels. There is no evidence that dogs or cats develop sufficient viremia to become amplifier hosts (Austgen et al. 2004).

References

Austgen LE, Bowen RA, Bunning ML, Davis BS, Mitchell CJ, Chang GJ. 2004. Experimental infection of cats and dogs with West Nile virus. *Emerg Inf Dis.* 10(1)82-86.

Carney RM, Ahearn SC, McConchie A, Glasner C, Jean C, Barker C, Park B, Padgett K, Parker E, Aquino E,Kramer V. 2011. Early warning system for West Nile virus risk areas, California, USA. *Emerg Infect Dis.* 7(8):1445-54.

CDC. 2016. Species of dead birds in which West Nile virus has been detected, United States, 1999-2016. https://www.cdc.gov/westnile/resources/pdfs/BirdSpecies1999-2016.pdf [PDF – 4 pages]

Docherty DE, Long RR, Griffin KM, Saito EK. 2004. Corvidae feather pulp and West Nile virus detection. *Emerg Infect Dis.* 10(5):907-9.

Eidson M. 2001a. "Neon needles" in a haystack: the advantages of passive surveillance for West Nile virus. *Ann N Y Acad Sci.* 951:38-53.

Eidson M, Schmit K, Hagiwara Y, Anand M, Backenson PB, Gotham I. 2001b. Dead crow densities and human cases of West Nile virus, New York State. *Emerg Infect Dis.* 7:662–4.

Fonseca K, Prince GD, Bratvold J, Fox JD, Pybus M, Preksaitis JK, Tilley P. 2005. West Nile virus infection and conjunctival exposure. *Emerg Infect Dis.* 11(10):1648-9.

Guptill SC, Julian KG, Campbell GL, Price SD, Marfin AA. 2003. Early-season avian deaths from West Nile virus as warnings of human infection. *Emerg Infect Dis.* 9(4):483-4.

Healy J, Reisen WK, Kramer V, Barker CM. 2012. Do current surveillance methods provide adequate warning for human infections with West Nile virus? *Proc Mosq Control Assoc Calif.* 80:17-21.

Johnson G, Nemeth N, Hale K, Lindsey N, Panella NA, Komar N. 2010. Surveillance for West Nile virus in American white pelicans, Montana, USA, 2006-2007. *Emerg Infect Dis.* 16(3):406–11.

Julian KG, Eidson M, Kipp AM, Weiss E, Petersen LR, Miller JR, Hinten SR, Marfin AA. 2002. Early season crow mortality as a sentinel for West Nile virus disease in humans, northeastern United States. *Vector Borne Zoonotic Dis.* 2(3):145-55.

Komar N. 2001. West Nile virus surveillance using sentinel birds. *Annals NY Acad Sci.* 951:58-73.

Komar N, Lanciotti R, Bowen R, Langevin S, Bunning M. 2002. Detection of West Nile virus in oral and cloacal swabs collected from bird carcasses. *Emerg Infect Dis.* 8(7):741-2.

Komar N. 2003. West Nile virus: epidemiology and ecology in North America. *Adv. Vir. Res.* 61:185-234.

Kwan JL, Kluh S, Madon MB, Nguyen DV, Barker CM, Reisen WK. 2010. Sentinel chicken seroconversions track tangential transmission of West Nile virus to humans in the greater Los Angeles area of California. *Am J Trop Med Hyg.* 83(5):1137-45.

Kwan JL, Park BK, Carpenter TE, Ngo V, Civen R, Reisen WK. 2012a. Comparison of enzootic risk measures for predicting West Nile disease, Los Angeles, California, USA, 2004-2010. *Emerg Infect Dis.* 18(8):1298-306.

Kwan JL, Kluh S, Reisen WK. 2012b. Antecedent avian immunity limits tangential transmission of West Nile virus to humans. *PLoS One.* 7(3):e34127.

Langevin SA, Bunning M, Davis B, Komar N. 2001. Experimental infection of chickens as candidate sentinels for West Nile virus. *Emerg Infect Dis* 7(4):726-729.

Lim AK, Dunne G, Gurfield N. 2009. Rapid bilateral intraocular cocktail sampling method for West Nile virus detection in dead corvids. *J Vet Diagn Invest.* 21(4):516-9.

Mattar S, Komar N, Young G, Alvarez J, Gonzalez M. 2011. Seroconversion to West Nile and St. Louis encephalitis viruses among sentinel horses, Colombia. *Memorias do Instituto Oswaldo Cruz.* 106(8): 976-79.

Mostashari F, Kulldorff M, Hartman JJ, Miller JR, Kulasekera V. 2003. Dead bird clusters as an early warning system for West Nile virus activity. *Emerg Infect Dis.* 9:641-646. Doi: 10.3201/eid0906.020794

Nemeth N, Beckett S, Edwards E, Klenk K, Komar N. 2007a. Avian mortality surveillance for West Nile virus in Colorado. *Am J Trop Med Hyg.* 76:431-7.

Nemeth N, Kratz G, Edward E, Scherpelz J, Bowen R, Komar N. 2007b. Evaluation of clinic-admitted raptors for West Nile virus surveillance. *Emerg Infect Dis.* 13(2): 305-307.

Nemeth NM, Burkhalter KL, Young GR, Brault AC, Reisen WK, Komar N. 2009. West Nile virus detection in nonvascular feathers from avian carcasses. *J Vet Diagn Invest.* 21:616–622.

Panella NA, Kerst AJ, Lanciotti RS, Bryant P, Wolf B, Komar N. 2001. Comparative West Nile virus detection in organs of naturally infected American crows (*Corvus brachyrhynchos*). *Emerg Infect Dis.* 7(4):754-5.

Patnaik JL, Juliusson L, Vogt RL. 2007. Environmental predictors of human West Nile virus infections, Colorado. *Emerg Infect Dis.* 13(11):1788-90.

Phoutrides E, Jusino-Mendez T, Perez-Medina T, Seda-Lozada R, Garcia-Negron M, Davila-Toro F, Hunsperger E. 2011. The utility of animal surveillance in the detection of West Nile virus activity in Puerto Rico, 2007. *Vector Borne Zoonotic Dis.* 11(4):447-50.

Reed LM, Johannson MJ, Panella N, McLean RG, Creekmore T, Puelle R, Komar N. 2009. Declining mortality in American crow (*Corvus brachyrhynchos*) following natural West Nile virus infection. *Avian Dis.* 53:458–61.

Resnick MP, Grunenwald P, Blackmar D, Hailey C, Bueno R, Murray KO. 2008. Juvenile dogs as potential sentinels for West Nile virus surveillance. *Zoonoses Public Health.* 55(8-10):443-7

Unlu I, Roy AF, Yates M, Garrett D, Bell H, Harden T, Foil LD. 2009. Evaluation of surveillance methods for detection of West Nile virus activity in East Baton Rouge Parish, Louisiana, 2004-2006. *J Am Mosq Control Assoc.* 25(2):126-33. Doi:10.2987/08-5713.1

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Last Reviewed: June 6, 2022

Epidemiology and Ecology

Epidemiology and Ecology

West Nile virus (WNV), a mosquito-transmitted member of the genus *Flavivirus* is the most frequent cause of arboviral disease in the continental United States and is recognized as the most widely distributed arbovirus in the world (Kramer et al. 2008). First identified in northwest Uganda in 1937 (Smithburn et al. 1940), WNV was not viewed as a public health threat until it was associated with epidemics of fever and encephalitis in the Middle East in the 1950s (Taylor et al. 1956). WNV caused only sporadic outbreaks of human disease globally until the mid-1990s, when frequent outbreaks began to occur in the Mediterranean Basin and large outbreaks in Romania and the Volga delta in southern Russia (Hayes et al. 2005).

The first domestically acquired human cases of WNV disease in the Western Hemisphere were detected in New York City in 1999 (Nash et al. 2001). WNV rapidly spread during the following years and by 2005 had established sustained transmission foci in much of the hemisphere with an overall distribution that extended from central Canada to southern Argentina (Gubler 2007).

WNV disease cases have been reported from all 48 contiguous states and two-thirds of U.S. counties. During the first 10 years after WNV was first detected in the United States in 1999, the annual incidence of neuroinvasive disease fluctuated considerably. However, during more recent years, the national incidence of neuroinvasive disease has been relatively stable at around 0.44 per 100,000 population (McDonald et al. 2021). Despite this stability, the occurrence of WNV disease cases continues to be focal and sporadic in nature when assessed at the state and county levels. Annual incidence of WNV disease is most often high in the West Central and Mountain regions, with the highest cumulative incidence of infection in the central plains states (i.e., South Dakota, Wyoming, and North Dakota) (Petersen et al. 2012, McDonald et al. 2021). The greatest disease burden occurs where areas of moderate to high incidence intersect metropolitan counties with high human population densities.

Human WNV disease cases have occurred every month of the year in the United States. However, transmission is highest in summer and early fall, with 94% of human cases reported from July through September and approximately two-thirds of cases in a 6-week period from mid-July through the end of August (McDonald et al. 2021). Weather, especially temperature, is an important modifier of WNV transmission, and has been correlated with increased incidence of human disease at regional and national scales (Soverow et al. 2009).

WNV is primarily maintained in an enzootic transmission cycle between *Culex* species mosquitoes and birds as the vertebrate hosts. Epidemic (and epizootic) transmission occurs when the virus escapes the bird-to-bird enzootic cycle to infect other vertebrates, including humans. In the US, WNV is enzootic in all 48 contiguous United States and evidence of transmission in the form of infected humans, mosquitoes, birds, horses, or other mammals has been reported from 96% of U.S. counties. Though WNV has been detected in 65 different mosquito species in the United States (CDC 2021), only a few *Culex* species drive epizootic and epidemic transmission. The most important vectors are *Cx. pipiens* in the northern states, *Cx. quinquefasciatus* in the southern states, and *Cx. tarsalis* in the western states where it overlaps with the *Cx. pipiens* and *Cx. quinquefasciatus* (Figure) (Andreadis et al. 2004, Kilpatrick et al. 2006a, Godsey et al. 2012).

Across middle latitudes of the United States, *Cx. pipiens* and *Cx. quinquefasciatus* are present both as nominal species and hybrids and are commonly reported as *Cx. pipiens* complex mosquitoes (Savage and Kothera 2012). *Culex salinarius* is an important enzootic and epidemic vector in the northeastern United States (Anderson et al. 2004, 2012,

Molaei et al. 2006). Other mosquito species including *Cx. restuans, Cx. nigripalpus,* and *Cx. stigmatosoma* may contribute to early season amplification or serve as bridge vectors, feeding on both birds and mammals and potentially contributing to human infection (Kilpatrick et al. 2005).

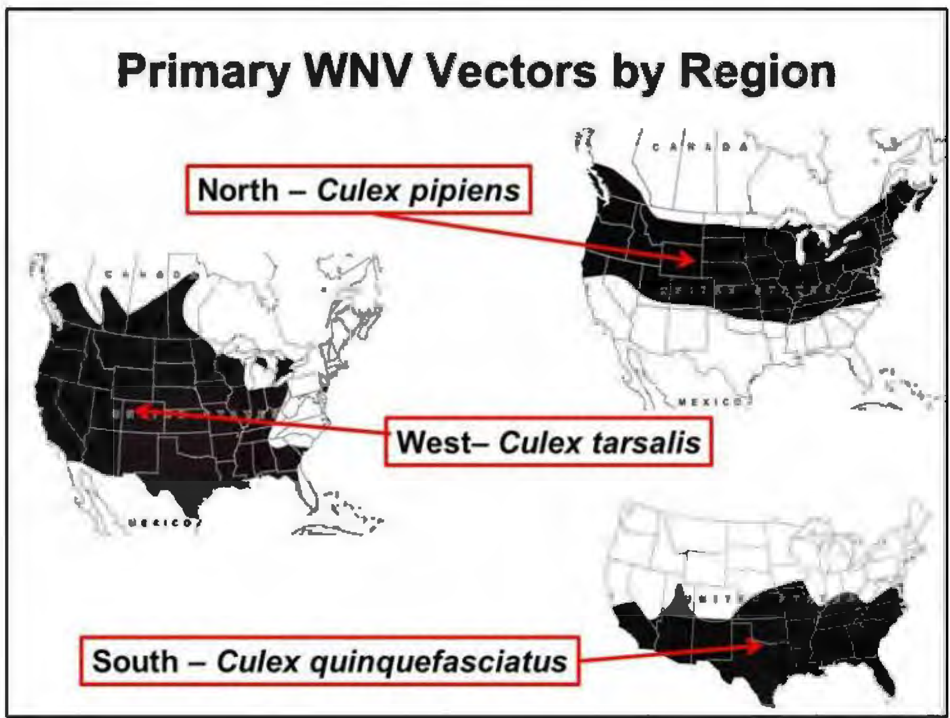

Figure. Approximate geographic distribution of the primary WNV vectors, *Cx. pipiens, Cx. quinquefasciatus,* and *Cx. tarsalis* (modified from Darsie and Ward 2005).

WNV has been detected in hundreds of bird species in the United States (CDC 2021) but only a few are primary amplifiers of the virus and influence WNV transmission locally (Hamer et al. 2009). Passerine birds typically are involved in West Nile virus amplification in many locations. For example, the American robin (*Turdus migratorious*) can be an amplifier host even in locations where it is present in low abundance (Kilpatrick et al. 2006b). Some infected birds, especially crows and jays, are known to get sick and die from the infection.

References

Anderson JF, Andreadis TG, Main AJ, Kline DL. 2004. Prevalence of West Nile virus in tree canopy-inhabiting *Culex pipiens* and associated mosquitoes. *Am J Trop Med Hyg.* 71(1):112-9.

Anderson JF, Main AJ, Cheng G, Ferrandino FJ, Fikrig E. 2012. Horizontal and vertical transmission of West Nile virus genotype NY99 by *Culex salinarius* and genotypes NY99 and WN02 *by Culex tarsalis. Am J Trop Med Hyg.* 86(1):134-9.

Andreadis TG, Anderson JF, Vossbrinck CR, et al. 2004. Epidemiology of West Nile virus in Connecticut: A five-year analysis of mosquito data 1999–2003. *Vector-Borne Zoonotic Dis.* 4:360–378.

CDC. 2021. West Nile virus: Mosquito Control. Key Resources for Professionals. https://www.cdc.gov/westnile/vectorcontrol/index.html. Accessed 7/14/2021.

Darsie RF, Ward RA. 2005. Identification and Geographical Distribution of the Mosquitoes of North America, North of Mexico. University of Florida Press, Gainesville, FL. 383 pp.

Godsey MS Jr., Burkhalter K, Young G, Delorey M, Smith K, Townsend J, Levy C, Mutebi JP. 2012. Entomologic investigations during an outbreak of West Nile virus disease in Maricopa County, Arizona, 2010. *Am J Trop Med Hyg.* 87(6):1125-1131.

Gubler, DJ. 2007. The Continuing Spread of West Nile Virus in the Western Hemisphere. *Clinical Inf Dis.* 45:1039–46 51

Hamer GL, Kitron UD, Goldberg TL, Brawn JD, Loss SR, Ruiz OR, et al. 2009. Host selection by *Culex pipiens* mosquitoes and West Nile virus amplification. *Am J Trop Med Hyg.* 80:268–278.

Hayes EB, Komar N, Nasci RS, Montgomery SP, O'Leary DR, Campbell GL, 2005. Epidemiology and transmission dynamics of West Nile virus disease. *Emerg Infect Dis.* 11: 1167–1173.

Kilpatrick AM, Kramer LD, Campbell SR, Alleyne EO, Dobson AP, Daszak P. 2005. West Nile virus risk assessment and the bridge vector paradigm. *Emerg Infect Dis.* 11(3):425-9.

Kilpatrick AM, Kramer LD, Jones MJ, Marra PP, Daszak P. 2006a. West Nile virus epidemics in North America are driven by shifts in mosquito feeding behavior. *PLoS Biol.* 4:606–610.

Kilpatrick AM, Daszak P, Jones MJ, Marra PP, Kramer LD. 2006b. Host heterogeneity dominates West Nile virus transmission. *Proc Biol Sci.* 273(1599):2327-33.

Kramer, LD, LM. Styer,GD. Ebel. 2008. A Global Perspective on the Epidemiology of West Nile Virus. *Ann Rev Entomol.* 53:61–81

McDonald ES, Mathis S, Martin SW, Staples JE, Fischer M, Lindsey NP. 2021.Surveillance for West Nile virus disease — United States, 2009–2018. *MMWR Morb Mortal Wkly Rep*;70(1):1-15.

Molaei G, Andreadis TG, Armstrong PM, Anderson JF, Vossbrinck CR. 2006. Host feeding patterns of *Culex* mosquitoes and West Nile virus transmission, northeastern United States. *Emerg Infect Dis.* 12(3):468-74.

Nash DMF, Fine A, Miller J, O'Leary D, Murray K, Huang A. 2001. The outbreak of West Nile virus infection in the New York City area in 1999. *N Engl J Med.* 344: 1807–1814.

Petersen LR, Carson PJ, Biggerstaff BJ, Custer B, Borchardt SM, Busch MP. 2012. Estimated cumulative incidence of West Nile virus infection in US adults, 1999-2010. *Epidemiol Infect.* 1-5.

Savage HM, Kothera L. 2012. The *Culex pipiens* complex in the Mississippi River basin: identification, distribution, and bloodmeal hosts. *J Am Mosq Control Assoc.* 28(4 Suppl):93-9.

Smithburn KC, Hughes TP, Burke AW, Paul JH.1940. A neurotropic virus isolated from the blood of a native of Uganda. *Am J Trop Med Hyg.* 20:471-92.

Soverow JE, Wellenius GA, Fisman DN, Mittleman MA. 2009. Infectious disease in a warming world: how weather influenced West Nile virus in the United States (2001-2005). *Environmental Health Perspect.* 117(7):1049-1052.

Taylor RM, Work TH, Hurlbut HS, Rizk F. 1956. A study of the ecology of West Nile virus in Egypt. *Am J Trop Med Hyg.* 5:579-620. 58

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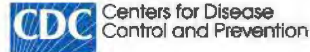

Mosquitoes

Human Disease

Routes of Transmission

West Nile virus (WNV) is transmitted to humans primarily through the bite of infected mosquitoes (Campbell et al. 2002). However, person-to-person transmission can occur through transfusion of infected blood products or solid organ transplantation (Pealer et al. 2003, Iwamoto et al. 2003). Intrauterine transmission and probable transmission via human milk also have been described but appear to be uncommon (O'Leary et al. 2006, Hinckley et al. 2007). Percutaneous infection and aerosol infection have occurred in laboratory workers, and an outbreak of WNV infection among turkey handlers also raised the possibility of aerosol transmission (CDC 2002, CDC 2003a).

Since 2003, the U.S. blood supply has been routinely screened for WNV RNA; as a result, transfusion associated WNV infection is rare (CDC 2003b). The U.S. Food and Drug Administration (FDA) recommends that blood collection agencies perform WNV nucleic acid amplification test (NAAT) year-round on all blood donations, either in minipools of six or 16 donations (depending on test specifications) or as individual donations. Organ and tissue donors are not routinely screened for WNV infection though a few collection agencies have incorporated screening of donors (Nett et al. 2012, Theodoropoulos et al. 2021).

Clinical Presentation and Evaluation

An estimated 70-80% of human WNV infections are subclinical or asymptomatic (Mostashari et al. 2001, Zou et al. 2010). Most symptomatic persons experience an acute systemic febrile illness that often includes headache, myalgia, or arthralgia; gastrointestinal symptoms and a transient maculopapular rash also are commonly reported (Watson et al. 2004, Hayes et al. 2005, Zou et al. 2010). Less than 1% of infected persons develop neuroinvasive disease, which typically manifests as meningitis, encephalitis, or acute flaccid paralysis (Hayes et al. 2005). WNV meningitis is clinically indistinguishable from aseptic meningitis due to most other viruses (Sejvar and Marfin 2006). Patients with WNV encephalitis usually present with seizures, mental status changes, focal neurologic deficits, or movement disorders (Sejvar and Marfin 2006). WNV acute flaccid paralysis is often clinically and pathologically identical to poliovirus-associated poliomyelitis, with damage of anterior horn cells, and may progress to respiratory paralysis requiring mechanical ventilation (Sejvar and Marfin 2006). WNV-associated Guillain-Barré syndrome has also been reported and can be distinguished from WNV poliomyelitis by clinical manifestations and electrophysiologic testing (Sejvar and Marfin 2006). Cardiac dysrhythmias, myocarditis, rhabdomyolysis, optic neuritis, uveitis, chorioretinitis, orchitis, pancreatitis, and hepatitis have been described rarely with WNV infection (Hayes et al. 2005).

Although people of all age groups appear to be equally susceptible to WNV infection, the incidence of neuroinvasive WNV disease increases with age (McDonald et al. 2021). In addition, among patients with neuroinvasive WNV disease, older adults are more likely to develop encephalitis or meningoencephalitis and have substantially higher case-fatality rates compared with children or younger adults. Solid organ transplant recipients also are at significantly higher risk of severe illness. Severe WNV disease has been described in persons with malignancies, but the relative risk from these or other immunocompromising conditions remains unclear. Hypertension, cerebrovascular disease, chronic renal disease, alcohol abuse, and diabetes mellitus also have been identified as possible risk factors for severe WNV disease, but further research is warranted (Murray et al 2006, Lindsey et al 2012).

The differential diagnosis of arboviral central nervous system disease is broad and includes many infectious (e.g., viral, bacterial, mycoplasmal, protozoal, or mycotic) and noninfectious (e.g., toxic, metabolic, or postinfectious) causes. Other viral causes of acute neurological illness include herpes simplex, enterovirus, rabies, measles, mumps, Epstein-Barr,

varicella zoster, and influenza viruses.

References

Campbell GL, Marfin AA, Lanciotti RS, Gubler DJ, 2002. West Nile virus. *Lancet Infect Dis.* 2: 519-29. 49

CDC. 2002. Laboratory-acquired West Nile virus infections—United States, 2002. *MMWR Morb Mortal Wkly Rep.* 51: 1133-5.

CDC. 2003a. West Nile virus infection among turkey breeder farm workers—Wisconsin, 2002. *MMWR Morb Mortal Wkly Rep.* 52: 1017-9.

CDC. 2003b. Detection of West Nile virus in blood donations—United States, 2003. *MMWR Morb Mortal Wkly Rep.* 52: 769-72.

Hayes EB, Sejvar JJ, Zaki SR, Lanciotti RS, Bode AV, Campbell GL. 2005. Virology, pathology, and clinical manifestations for West Nile virus disease. *Emerg Infect Dis.* 11: 1174-9.

Hinckley AF, O'Leary DR, Hayes EB. 2007. Transmission of West Nile virus through human breast milk seems to be rare. *Pediatrics.* 119(3): e666-71.

Iwamoto M, Jernigan DB, Guasch A, et al. 2003. Transmission of West Nile virus from an organ donor to four transplant recipients. *N Eng J Med.* 348(22): 2196-203.

Lindsey NP, Staples JE, Lehman JA, Fischer M. 2012. Medical risk factors for severe West Nile virus disease, United States, 2008-2010. *Am J Trop Med Hyg.* 87(1):179-84.

McDonald ES, Mathis S, Martin SW, Staples JE, Fischer M, Lindsey NP. 2021. Surveillance for West Nile virus disease — United States, 2009–2018. *MMWR Morb Mortal Wkly Rep* ;70(1):1-15.

Mostashari F, Bunning ML, Kitsutari PT, et al. 2001. Epidemic West Nile encephalitis, New York, 1999: results of a household-based seroepidemiological survey. *Lancet.* 358: 261-4. 55

Murray K, Baraniuk S, Resnick M, et al. Risk factors for encephalitis and death from West Nile virus infection. *Epidemiol Infect* 2006; 134:1325–32.

Nett RJ, Kuehnert MJ, Ison MG, Orlowski JP, Fischer M, Staples JE. 2012. Current practices and evaluation of screening solid organ donors for West Nile virus. *Transpl Infect Dis.* 14(3):268-77.

O'Leary DR, Kuhn S, Kniss KL, et al. 2006. Birth outcomes following West Nile virus infection of pregnant women in the United States: 2003-2004. *Pediatrics.* 117(3): e537-45.

Pealer LN, Marfin AA, Petersen LR, et al. 2003. Transmission of West Nile virus through blood transfusion in the United States in 2002. *N Engl J Med.* 349(13):1236-45.

Sejvar JJ, Marfin AA. 2006. Manifestations of West Nile neuroinvasive disease. *Rev Med Virol.* 16(4): 209-24.

Theodoropoulos NM, Greenwald MA, Chin-Hong P, Ison MG. 2021. Testing deceased organ donors for infections: An organ procurement organization survey. *Am J Transplant.* May;21(5):1924-1930.

Watson JT, Pertel PE, Jones RC, et al. 2004. Clinical characteristics and functional outcomes of West Nile fever. *Ann Intern Med.* 141: 360-5.

Zou S, Foster GA, Dodd RY, Petersen LR, Stramer SL. 2010. West Nile fever characteristics among viremic persons identified through blood donor screening. *J Inf Dis.* 202:1354-1361.

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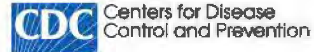

Human Laboratory Diagnosis and Testing

A Laboratory Diagnosis of Human Arboviral Diseases

Laboratory testing for evidence of arboviral diseases typically involves serologic and molecular testing. For several viruses where humans are an amplifying host, molecular testing is more specific and can be used to confirm the diagnosis in the first week of illness. For viruses that typically are neuroinvasive, serology is more likely to be used to determine if someone was recently infected.

In most patients, infection with an arbovirus that can cause encephalitis is clinically inapparent or causes a nonspecific viral syndrome. Numerous pathogens cause encephalitis, aseptic meningitis, and febrile disease with similar clinical symptoms and presentations and should be considered in the differential diagnosis. Definitive diagnosis can only be made by laboratory testing using specific reagents. Selection of diagnostic test procedures should take into consideration patient factors (e.g., age, immune status, vaccination history), timing of infection, the range of pathogens in the differential diagnosis, the criteria for classifying a case as confirmed or probable, as well as the capability of the primary and confirming diagnostic laboratories.

Appropriate selection of diagnostic procedures and accurate interpretation of findings requires information describing the patient and the diagnostic specimen. For human specimens, the following data must accompany sera, CSF or tissue specimens for results to be properly interpreted and reported: 1) symptom onset date (when known); 2) date of sample collection; 3) unusual immunological status of patient (e.g., immunosuppression); 4) state and county of residence; 5) travel history (especially in flavivirus-endemic areas); 6) history of prior vaccination (e.g., yellow fever, Japanese encephalitis, or tick-borne encephalitis viruses); and 7) brief clinical summary including clinical diagnosis (e.g., encephalitis, aseptic meningitis). Minimally, onset and sample collection dates are required to perform and interpret initial screening tests. The remaining information is required to evaluate any test results from initial screening. If possible, a convalescent serum sample taken at least 14 days following the acute sample should be obtained to enable confirmation by serological testing.

Human Diagnostic Testing

Serology. The front-line screening assay for laboratory diagnosis of human WNV infection is the IgM assay. Currently, the FDA has cleared three commercially available test kits from different manufacturers, for detection of WNV IgM antibodies. These kits are used in many commercial and public health laboratories in the United States. In addition, the CDC-defined IgM and IgG EIA [i.e., ELISA or microsphere-based immunoassay (MIA)] can be used (Martin et al. 2000; Johnson et al. 2000; Johnson et al. 2005). The CDC MIA can differentiate WNV from St. Louis encephalitis (SLE). Protocols are available for the CDC-developed assays from CDC's DVBD Diagnostic Laboratory (Martin et al. 2000; Johnson et al. 2000). CDC also will provide positive controls and limited reagents as commercial sources are available to state public health labs.

Because the IgM and IgG antibody tests can cross-react between flaviviruses (e.g., SLE, dengue, yellow fever, WNV, Powassan), they should be viewed as screening tests only. For a case to be considered confirmed, serum samples that are antibody-positive on initial screening should be evaluated by a more specific test; currently the plaque reduction neutralization test (PRNT) is the recommended test for differentiating between flavivirus infections. Though WNV is the

most common cause of arboviral encephalitis in the United States, there are several other arboviral encephalitides present in the country and in other regions of the world. Specimens submitted for WNV testing should also be tested against other arboviruses known to be active or be present in the area or in the region where the patient traveled.

Virus Detection Assays. Numerous procedures have been developed for detecting viable WNV, WNV antigen, or WNV RNA in human diagnostic samples, many of which have been adapted to detecting WNV in other vertebrates and in mosquito samples. These procedures vary in their sensitivity, specificity, and time required to conduct the test (Table).

Test	Detects	Detection Level (pfu/ml)	Assay Time
Virus isolation in suckling mouse	Infectious virus	100	4-10 days
Virus isolation in cell culture	Infectious virus	100	3 days
Standard RT-PCR	Viral RNA	5	8 hours
Nucleic Acid Sequence Based Amplification (NASBA)	Viral RNA	0.1	4 hours
Real Time RT-PCR	Viral RNA	0.1	4 hours
Transcription Mediated Amplification	Viral RNA	0.02	4 hours

Resources for Human Diagnostic Laboratories

Clinical Laboratory Improvements Amendments (CLIA) certification: To maintain certification, CLIA recommendations for performing and interpreting human diagnostic tests should be followed. Laboratories performing arboviral serology or RNA-detection testing are invited to participate in the annual proficiency testing that is available from CDC's Division of Vector-Borne Diseases in Fort Collins, CO. To obtain additional information about the proficiency testing program and about training in arbovirus diagnostic procedures, contact the Division of Vector-Borne Diseases by phone: 970-261-6400 or email: dvbid2@cdc.gov.

Biocontainment: Containment specifications are available in the CDC/National Institutes of Health publication Biosafety in Microbiological and Biomedical Laboratories (BMBL 6). This document can be found online at: https://www.cdc.gov/csels/dls/locs/2020/cdc_releases_6th_edition_of_biosafety_in_microbiology_and_biomedial_labs.html

Shipping of diagnostic samples and agents. Shipping and transport of clinical specimens should follow current International Air Transport Association (IATA) and Department of Commerce recommendations. For more information, visit the IATA dangerous goods Web site at:

http://www.iata.org/publications/dgr/Pages/index.aspx, and the USDA Animal and Plant Health Inspection Service (APHIS), National Center for Imports and Exports website: https://www.aphis.usda.gov/aphis/ourfocus/importexport

References

Bhatnagar J, Guarner J, Paddock CD, Shieh WJ, Lanciotti RS, Marfin AA, Campbell GL, Zaki SR. 2007. Detection of West Nile virus in formalin-fixed, paraffin-embedded human tissues by RT-PCR: a useful adjunct to conventional tissue-based diagnostic methods. *J Clin Virol.* 38(2):106-11. Epub 2006 Dec 8. 48

Briese T, Glass WG, Lipkin WI.2000. Detection of West Nile virus sequences in cerebrospinal fluid. *Lancet.* 355:1614-5.

Johnson AJ, Martin DA, Karabatsos N, Roehrig JT. 2000. Detection of anti-arboviral immunoglobulin G by using a monoclonal antibody-based capture enzyme-linked immunosorbent assay. *J Clin Microbiol.* 38:1827-31.

Johnson AJ, Noga AJ, Kosoy O, Lanciotti RS, Johnson AA, Biggerstaff BJ. 2005. Duplex microsphere-based immunoassay for detection of anti-West Nile virus and anti-St. Louis encephalitis virus immunoglobulin m antibodies. *Clin Diagn Lab Immunol.* 12(5):566-74.

Lanciotti RS, Kerst AJ, Nasci RS, Godsey MS, Mitchell CJ, Savage HM, et al. 2000. Rapid detection of West Nile virus from human clinical specimens, field- collected mosquitoes, and avian samples by a TaqMan reverse transcriptase-PCR assay. *J Clin Microbiol.* 38:4066-71.

Martin DA, Muth DA, Brown T, Johnson AJ, Karabatsos N, Roehrig JT. 2000 Standardization of immunoglobulin M capture enzyme-linked immunosorbent assays for routine diagnosis of arboviral infections. *J Clin Microbiol.* 38:1823-6.

Shi PY, Kauffman EB, Ren P, Felton A, Tai JH, Dupuis AP. et al. 2001. High-throughput detection of West Nile virus RNA. *J Clin Microbiol.* 39:1264-71.

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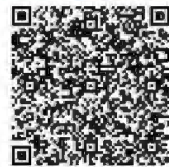
Last Reviewed: June 7, 2022



Mosquitoes

Human Surveillance

Passive Surveillance and Case Investigation

Arboviral diseases are nationally notifiable conditions and many are explicitly reportable U.S. states and territories. Most disease cases are reported to public health authorities from public health or commercial laboratories; healthcare providers can also directly submit reports of suspected cases. State and local health departments are responsible for ensuring that reported human disease cases meet the national case definitions. The most recent case definitions for arboviral diseases can be found on the CDC Nationally Notifiable Disease Surveillance System website (https://ndc.services.cdc.gov/case-definitions/). For some arboviruses (e.g., West Nile and Zika viruses) presumptive viremic donors are identified through universal screening of the blood supply; case definitions and reporting practices for viremic donors vary by jurisdiction and blood services agency.

All identified human disease cases and presumptive viremic blood donors should be investigated promptly. Jurisdictions may choose to interview the patient's healthcare provider, the patient, or both depending on information needs and resources. Whenever possible, the following information should be gathered:

- Basic demographic information (age, sex, race/ethnicity, state and county of residence)
- Clinical syndrome (e.g., asymptomatic blood donor, uncomplicated fever, meningitis, encephalitis, acute flaccid paralysis)
- Illness onset date and/or date of blood donation
- If the patient was hospitalized and if he/she survived or died
- Travel history in the four weeks prior to onset
- If the patient was an organ donor or a transplant recipient in the 4 weeks prior to onset
- If the patient was a blood donor or blood transfusion recipient in the 4 weeks prior to onset
- If the patient was pregnant at illness onset
- If the patient is an infant, was he/she breastfed before illness onset

If the patient donated blood, tissues or organs in the 4 weeks prior to illness onset, immediately inform the blood or tissue bank and appropriate public health authorities. Similarly, any infections temporally associated with blood transfusion or organ transplantation should be reported. Prompt reporting of these cases will facilitate the identification and quarantine of any remaining infected products and the identification of any other exposed recipients so they may be managed appropriately.

Passive surveillance systems are dependent on clinicians considering the diagnosis of an arboviral disease and obtaining the appropriate diagnostic test and reporting of laboratory-confirmed cases to public health authorities. Because of incomplete diagnosis and reporting, the incidence of arboviral diseases is underestimated. Where applicable, reported neuroinvasive disease cases are considered the most accurate indicator of activity in humans because of the substantial associated morbidity. In contrast, reported cases of non-neuroinvasive disease are more likely to be affected by disease awareness and healthcare-seeking behavior in different communities and by the availability and specificity of laboratory tests performed.

Enhanced Surveillance Activities

Enhanced surveillance for human arboviral disease cases should be considered when environmental or human surveillance suggests that an outbreak is suspected or anticipated. Educating healthcare providers and infection control nurses about the need for arbovirus testing and reporting of all suspected cases could increase the sensitivity of the surveillance system. This might be accomplished by distributing print materials, participating in local hospital meetings and grand rounds, and providing lectures/seminars. Public health agencies should also work to establish guidelines and protocols with local blood collection agencies for reporting viremic blood donors. At the end of the year, an active review of medical records and laboratory results from local hospitals and associated commercial laboratories should be conducted to identify any previously unreported cases. In addition, an active review of appropriate records from blood collection agencies could be conducted to identify any positive donors that were not reported.

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Last Reviewed: June 6, 2022

Non-human Laboratory Diagnosis

Laboratory Testing of Vectors

Identification and Pooling

Mosquitoes should be identified to species or lowest taxonomic unit. Specimens are placed into pools of 50 specimens or less based on species, sex, location, trap-type, and date of collection. Larger pool sizes can be used in some assays with loss of sensitivity (Sutherland and Nasci 2007). If resources are limited, testing of mosquitoes for surveillance purposes can be limited to the primary vector species.

Homogenizing and Centrifugation

After adding the appropriate media, mosquito pools can be macerated or ground by a variety of techniques including mortar and pestle, vortexing sealed tubes containing one or more copper clad BBs, or by use of tissue homogenizing apparatus that are commercially available (Savage et al. 2007). After grinding, samples are centrifuged, and an aliquot is removed for testing. Because mosquito pools may contain arboviruses and other pathogenic viruses, which may be aerosolized during processing, laboratory staff should take appropriate safety precautions including use of a Class II Type A biological safety cabinet and wearing appropriate personal protective equipment (PPE) and adhering to biosafety practices.

Virus Detection

Virus isolation in Vero cell culture remains the standard for confirmation of positive pools (Beaty et al. 1989, Savage et al. 1999, Lanciotti et al. 2000). Virus isolation provides the benefit of detecting other viruses that may be contained in the mosquitoes, a feature that is lost using test procedures that target virus-specific nucleotide sequence or proteins. However, Vero cell culture is expensive and requires specialized laboratory facilities; thus, nucleic acid assays have largely replaced virus isolation as detection and confirmatory assay methods of choice. Virus isolation requires that mosquito pools be ground in a media that protects the virus from degradation such as BA-1 (Lanciotti et al. 2000), and preservation of an aliquot at -70°C to retain virus viability for future testing.

Nucleic acid detection assays are the most sensitive assays for virus detection and confirmation of virus in mosquito pools (Lanciotti et al. 2000, Nasci et al. 2002). Real-Time RT-PCR assays with different primer sets may be used for both detection and confirmation of virus in mosquito pools. Standard RT-PCR primers are also available (Kuno et al 1998). Nucleic acids may be extracted from an aliquot of the mosquito pool homogenate by hand using traditional methods or with kits, or with automated robots in high-through-put laboratories (Savage et al 2007).

Virus antigen detection assays are available in ELISA format (Tsai et al. 1987, Hunt et al. 2002) and in commercial kits that employ lateral flow wicking assays, developed specifically for testing mosquitoes (Komar et al. 2002, Panella et al. 2001, Burkhalter et al. 2006). The antigen capture ELISA of Hunt et al. 2002 and the RAMP (Rapid Analyte Measurement Platform, Response Biomedical Corp, Burnaby, British Columbia, Canada) test are approximately equal in sensitivity and detect virus in mosquito pools at concentrations as low as $10^{3.1}$ PFU/ml (Burkhalter et al. 2006). The VecTest (Medical Analysis Systems, Inc., Camarillo, CA) is less sensitive and detects virus in mosquito pools at concentrations of $10^{5.17}$

PFU/ml. The VecTest (evaluated by Burkhalter et al. 2006) is no longer available but is similar to a lateral flow wicking assay marketed as VecTOR Test (VecTOR Test Systems, Inc., Thousand Oaks, CA). Although the antigen detection assays are less sensitive than nucleic acid detection assays, they have been evaluated in operational surveillance programs (Mackay et al. 2008. Lampman et al. 2006. Williges et al. 2009, Kesavaraju et al. 2012) and can provide valuable infection rate data when employed consistently in a mosquito surveillance program.

References

Burkhalter KL, Lindsay R, Anderson R, Dibernardo A, Fong W, Nasci RS. 2006. Evaluation of commercial assays for detecting West Nile virus antigen. *J Am Mosq Control Assoc.* 22:64-69.

Beaty BJ, Calisher CH, Shope RS. 1989 Arboviruses, p. 797-856. In Schmidt NJ, Emmons RW (ed.), Diagnostic procedures for viral, rickettsial and chlamydia infections. *American Public Health Assoc*, Washington DC.

Hunt AR, Hall RA, Kerst AJ, Nasci RS, Savage HM, Panella NA, et al. 2002. Detection of West Nile virus antigen in mosquitoes and avian tissues by a monoclonal antibody-based capture enzyme immunoassay. *J Clin Microbiol.* 40: 2023-30. 52

Kesavaraju B, Farajollahi A, Lampman RL, Hutchinson M, Krasavin NM, Graves SE, et al. 2012. Evaluation of a rapid analyte measurement platform for West Nile virus detection based on United States mosquito control programs. *Am J Trop Med Hyg.* 87(2):359-63.

Komar N, Lanciotti R, Bowen R, Langevin S, Bunning M. 2002. Detection of West Nile virus in oral and cloacal swabs collected from bird carcasses. *Emerg Infect Dis.* 8(7):741-2.

Kuno G, Chang G-J J, Tsuchiya KR, Karabatsos N, Cropp CB.1998. Phylogeny of the genus Flavivirus. *J Virol.* 72:73-83.

Lampman RL, Krasavin NM, Szyska M, Novak RJ. 2006. A comparison of two West Nile virus detection assays (TaqMan reverse transcriptase polymerase chain reaction and VecTest antigen assay) during three consecutive outbreaks in northern Illinois. *J Am Mosq Control Assoc.* 22(1):76-86.

Lanciotti RS, Kerst AJ, Nasci RS, Godsey MS, Mitchell CJ, Savage HM, et al. 2000. Rapid detection of West Nile virus from human clinical specimens, field- collected mosquitoes, and avian samples by a TaqMan reverse transcriptase-PCR assay. *J Clin Microbiol.* 38:4066-71.

Mackay AJ, Roy A, Yates MM, Foil LD. 2008. West Nile virus detection in mosquitoes in East Baton Rouge Parish, Louisiana, from November 2002 to October 2004. *J Am Mosq Control Assoc.* 24(1):28-35.

Nasci RS, Gottfried KL, Burkhalter KL, Kulasekera VL, Lambert AJ, Lanciotti RS, Hunt AR, Ryan JR. 2002. Comparison of vero cell plaque assay, TaqMan reverse transcriptase polymerase chain reaction RNA assay, and VecTest antigen assay for detection of West Nile virus in field-collected mosquitoes. *J Am Mosq Control Assoc.*18:294-300.

Panella NA, Kerst AJ, Lanciotti RS, Bryant P, Wolf B, Komar N. 2001. Comparative West Nile virus detection in organs of naturally infected American crows (*Corvus brachyrhynchos*). *Emerg Infect Dis.* 7(4):754-5.

Savage HM, Ceianu C, Nicolescu G, Karabatsos N, Lanciotti R, Vladimirescu A, et al. 1999. Entomologic and avian investigations of an epidemic of West Nile fever in Romania in 1996, with serologic and molecular characterization of a virus isolate from mosquitoes. *Am J Trop Med Hyg.* 61:600-11.

Savage HM, Aggarwal D, Apperson CS, Katholi CR, Gordon E, Hassan HK, Anderson M, Charnetzky D, McMillen L, Unnasch EA, Unnasch TR. 2007. Host choice and West Nile virus infection rates in blood-fed mosquitoes, including members of the *Culex pipiens* complex, from Memphis and Shelby County, Tennessee, 2002-2003. *Vector Borne Zoonotic Dis.* 7:365-86.

Sutherland GL, Nasci RS. 2007. Detection of West Nile virus in large pools of mosquitoes. *J Am Mosq Control Assoc.* 23:389-95.

Tsai TF, Bolin RA, Montoya M, Bailey RE, Francy DB, Jozan M, et al. 1987. Detection of St. Louis encephalitis virus antigen in mosquitoes by capture enzyme immunoassay. *J Clin Microbiol.* 25:370-6.

Williges E, Farajollahi A, Nelder MP, Gaugler R. 2009. Comparative field analyses of rapid analyte measurement platform and reverse transcriptase polymerase chain reaction assays for West Nile virus surveillance. *J Vector Ecol.* 34(2):324-8.

Laboratory Testing of Non-human Vertebrates

Serology

Diagnostic kits for serologic diagnosis of WNV infection in clinically ill domestic animals are not commercially available. IgM-capture ELISA has been developed for use in horses and can be readily adapted to other animal species where anti-IgM antibody reagents are commercially available. Alternatively, seroconversion for IgG, neutralizing antibodies, and haemagglutinin inhibiting (HAI) assays in acute and convalescent serum samples collected 2-3 weeks apart can be used as screening assays. The latter two approaches do not require species-specific reagents and thus have broad applicability. The ELISA format may be used when employed as inhibition or competition ELISAs, which avoids the use of species-specific reagents. A popular blocking ELISA has been applied to a variety of vertebrate species with very high specificity and sensitivity, reducing the necessity of a second confirmatory test (Blitvich et al 2003a, 2003b). Similarly, the microsphere immunoassay, when used comparatively with WNV antigen-coated beads and St. Louis encephalitis virus (SLEV) antigen-coated beads, performs with high specificity and sensitivity (Johnson et al. 2005). Typically, a confirmatory 90% plaque-reduction neutralization test ($PRNT_{90}$) with end-point titration is used to confirm serology in non-human vertebrates. Plaque-reduction thresholds below 80% are not recommended. Because of the cross-reactive potential of anti-flavivirus antibodies, the PRNT must be comparative, performed simultaneously with SLEV.

PRNTs require the use of a biosafety cabinet within a containment laboratory utilizing Vero cell culture. As of 2020, WNV was recommended in the Biomedical and Microbiological Laboratory guide (BMBL; v6 Biosafety in Microbiological and Biomedical Laboratories (BMBL) 6th Edition | CDC Laboratory Portal | CDC) to be handled under BSL-2 standards. Modification to the standard PRNT using a recombinant chimeric virus featuring the WNV envelope glycoprotein gene in a yellow fever virus backbone (Chimeravax®, originally developed as a live-attenuated vaccine candidate) can be used for an increased safety profile for lab staff. For PRNTs, the Chimeravax provided equivalent results for bird sera, and 10-100 fold lower titers for equine sera (Komar et al. 2009).

The same serologic techniques applied to clinically ill animals may also be used for healthy subjects for vertebrate serosurveys or for healthy sentinel animals serially sampled as sentinels. Serologic techniques for WNV diagnosis should not be applied to carcasses, as in many cases of fatal WNV infection, the host will die before a detectable immune response develops. Furthermore, some morbid or moribund animals that have WNV antibodies due to past infection may be currently infected with a pathogen other than WNV. Fatal cases should have readily detectable WNV in their tissues.

As with human diagnostic samples, serologic results from non-human vertebrates must be interpreted with caution and with an understanding of the cross-reactive tendencies of WNV and other flaviviruses. For primary WNV infections, a low rate of cross-reactivity is expected (<5%) and misdiagnoses are avoided by the requirement that the reciprocal anti-WNV titer be a minimum of 4-fold greater than the corresponding anti-SLEV titer. In rare cases, a secondary flavivirus infection due to WNV in a host with a history of SLEV infection may boost the older anti-SLEV titer to greater levels than the anti-WNV titer, resulting in a misdiagnosis of SLEV infection, a phenomenon known as "original antigenic sin". Some serum samples will have endpoint titers for WNV and SLEV that are the same or just 2-fold different. While it is possible that this serologic result is due to past infections with both of these viruses, it is impossible to rule out cross-reaction from one or the other, or even from a third indeterminate flavivirus. Such a result should be presented as "undifferentiated flavivirus infection."

Virus Detection

Methods for WNV detection, isolation, and identification are the same as described for human and mosquito diagnostics. Specimens typically used are tissues and/or fluids from acutely ill and/or dead animals. Virus detection in apparently healthy animals is very low-yield and inefficient, and therefore not cost-effective, and should not be considered for routine surveillance programs. In bird, mammal, and reptile carcasses, tissue tropisms have varied among individuals within a species, and across species. Some animals, like humans, have few tissues with detectable virus particles or viral RNA at necropsy, such as horses. Others, such as certain bird species, may have fulminant infections with high viral loads in almost every tissue.

References

Blitvich BJ, Marlenee NL, Hall RA, Calisher CH, Bowen RA, Roehrig JT. 2003a. Epitope-blocking enzyme-linked immunosorbent assays for the detection of serum antibodies to west nile virus in multiple avian species. *J Clin Microbiol.* 41(3):1041-7.

Blitvich BJ, Bowen RA, Marlenee NL, Hall RA, Bunning ML, Beaty BJ. 2003b. Epitope-blocking enzyme-linked immunosorbent assays for detection of west nile virus antibodies in domestic mammals. *J Clin Microbiol.* 41(6):2676-9.

Johnson AJ, Noga AJ, Kosoy O, Lanciotti RS, Johnson AA, Biggerstaff BJ. 2005. Duplex microsphere-based immunoassay for detection of anti-West Nile virus and anti-St. Louis encephalitis virus immunoglobulin m antibodies. *Clin Diagn Lab Immunol.* 12(5):566-74.

Komar, N., Langevin, S., Monath, T. P. 2009. Use of a surrogate chimeric virus to detect West Nile virus-neutralizing antibodies in avian and equine sera. *Clinical and Vaccine Immunology*, 16(1), 134-135.

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Last Reviewed: June 7, 2022

Objectives of Surveillance

Objectives of Arboviral Surveillance

Arboviral surveillance consists of two distinct, but complementary activities. Epidemiological surveillance measures human disease to quantify disease burden, detect early signs of an outbreak and identify information needed for timely responses, including seasonal, geographic, and demographic patterns in human morbidity and mortality. Environmental surveillance monitors local mosquito populations, virus activity in vectors and non-human vertebrate hosts, and other relevant environmental parameters to predict human risk and prevent outbreaks of arboviral disease in humans.

In addition to monitoring disease burden and distribution, epidemiological surveillance has been instrumental in characterizing clinical disease presentation and disease outcome, as well as identifying high-risk populations and factors associated with serious disease. Epidemiological surveillance has also detected and quantified alternative routes of transmission to humans, such as contaminated blood donations and organ transplantation.

Epidemiological and environmental surveillance for arboviruses is facilitated by ArboNET, the national arbovirus surveillance system. ArboNET was developed in 2000 as a comprehensive surveillance data capture platform to monitor West Nile virus (WNV) infections in humans, mosquitoes, birds, and other animals. This comprehensive approach was essential to tracking the progression of WNV as it spread and became established across the United States, and it remains a significant source of data on the epidemiology and ecology of WNV. Since 2003, ArboNET has also collected data on other domestic and exotic arboviruses of public health significance.

In the absence of effective human vaccines for most domestic arboviruses, preventing arboviral disease in humans primarily depends on measures to keep infected vectors from biting people. A principal objective of environmental surveillance is to quantify the intensity of virus transmission in a region and provide a predictive index of human infection risk. This risk prediction, along with information about the local conditions and habitats that impact vector abundance and infection, can be used to inform an integrated vector management program and decisions about implementing interventions to control mosquitoes and prevent disease.

Though epidemiological surveillance is essential for understanding arboviral disease burden, utilizing human case surveillance by itself is insufficient for predicting outbreaks. Outbreaks can develop quickly, with most human cases occurring over a few weeks during the peak of transmission. The time from human infection to onset of symptoms to diagnosis and reporting can be several weeks or longer. As a result, human case reports typically lag well behind the transmission from vectors that initiated the infection. By monitoring infection prevalence in vectors and incidence in non-human vertebrate hosts and comparing these indices to historical environmental and epidemiological surveillance data, conditions associated with increasing human risk can be detected 2-4 weeks in advance of human disease onset. This provides additional lead time for critical vector control interventions and public education programs to be put in place. The following sections describe the elements of epidemiological and environmental arboviral surveillance and how they may be used to monitor and predict risk and to trigger interventions.

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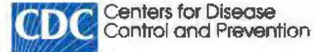

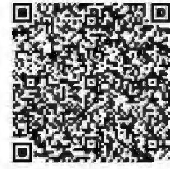

Mosquitoes

Prevention and Control: Community Engagement

Individual-Level Actions to Reduce Risk

Without an available, effective vaccine for people, the best way to prevent mosquito-borne disease is by preventing mosquito bites. This can be accomplished through community-based IVM programs and by personal protection behaviors, such as

- **Mosquito-avoidance.** Health officials may recommend residents avoid outdoor activities when mosquitoes are most active and high virus activity levels have been detected.
- **Use of personal repellents.** CDC recommends using EPA-registered insect repellents or covering up with long-sleeved shirts and long pants when outside.
- **Removal of residential mosquito sources.** Once a week, residents should empty, cover, or throw out items that hold water, such as tires, buckets, planters, toys, pools, birdbaths, flowerpots, or trash containers.

Jurisdictions can promote individual and community-based prevention measures through public education and risk communication activities. Messages should acknowledge the seriousness of the disease without promoting undue fear or panic in the target population. Fear-driven messages may heighten the powerlessness people express in dealing with vector-borne diseases. Messages should be clear and consistent with the recommendations of coordinating agencies and include a call to action. Use plain language and adapt materials for lower literacy and non-English speaking audiences.

Mosquito bites can be avoided simply by not going outdoors when mosquitoes are biting, and recommendations to avoid outdoor activity when and where high virus activity levels have been detected are a component of prevention programs. Recommendations to avoid being outdoors during peak mosquito biting times may conflict with neighborhood social patterns, community events, or the practices of persons without air-conditioning. It is important to communicate when the important mosquito vectors are most active. Emphasize that insect repellent use is protective and should be used when outdoors, particularly during the prime mosquito-biting hours.

Additional information about personal insect repellents, including permethrin, can be found at: https://www.cdc.gov/mosquitoes/mosquito-bites/prevent-mosquito-bites.html.

Information for individuals on control mosquitoes around their home can be found at: https://www.cdc.gov/mosquitoes/mosquito-control/athome/outside-your-home/index.html.

Prevention Strategies for High-Risk Groups

Audience members have different disease-related concerns and motivations for action. Proper message targeting (including use of plain language) permits better use of limited communication and prevention resources. The following are some population segments that require specific targeting. See Human Disease Section for additional information about risk groups that should be targeted.

Persons with Outdoor Exposure. People who engage in extensive outdoor work or recreational activities are at greater risk of being bitten by mosquitoes and ticks. Messages for these individuals should encourage use of insect repellent and protective clothing, particularly if outdoor activities occur during dusk to dawn hours. Local spokespersons (e.g., union

officials, job-site supervisors, golf pros, sports organizations, lawn care professionals, public works officials, gardening experts) may be useful collaborators. Place messages in locations where people engage in outdoor activities (e.g., parks, golf courses, hiking trails).

Homeless Populations. Extensive outdoor exposure and limited financial resources in this group present special challenges. Application of insect repellents to exposed skin and clothing may be most appropriate prevention measures for this population. Work with social service groups in your area to educate and provide insect repellents to this population segment.

Residences Lacking Window Screens. The absence of intact window/door screens is a likely risk factor for exposure to mosquito bites. Focus attention on the need to repair screens and provide access to resources to do so. Partner with community organizations that can assist older persons or others with financial or physical barriers to screen installation or repair.

Older Adults. For many mosquito-borne diseases, older adults are at greater risk for serious disease. Messages on mosquito avoidance, insect repellent use, and removal of mosquito sources around the home should be shared with this audience.

Communication and Community Engagement

At the community level, reporting dead birds and nuisance mosquito problems, advocating for organized mosquito abatement, and participating in community mobilization projects to address sources of mosquitoes such as trash, standing water or neglected swimming pools are activities that can help protect individuals and at-risk groups.

Providing clear messages and understandable concepts promotes community understanding and acceptance. The following provides a description of selected best practices for reaching high-risk groups, offers suggestions for cultivating partnerships with media and communities, and provides select outreach measures for mobilizing communities.

Communicating About Vector Control

Public understanding and acceptance of emergency adult mosquito control operations using insecticides is critical to its success, especially where these measures are unfamiliar. Questions about the products being used, their safety, and their effects on the environment are common. Improved communication about surveillance and how decisions to use mosquito adulticides are made may help residents weigh the risks and benefits of control. When possible, provide detailed information regarding the schedule for adulticiding through newspapers, radio, government-access television, the internet, recorded phone messages, social media or other means your agency uses to successfully communicate with its constituencies.

Community Mobilization and Outreach

Community mobilization can improve education and help achieve behavior change goals. Promote the concept that health departments and mosquito control programs require community assistance to reduce mosquito-borne disease risk. Leverage online platforms to further disseminate your messages.

A community task force that includes civic, business, public health, and environmental concerns can be valuable in achieving buy-in from various segments of the community, and in developing a common message. Community mobilization activities can include clean-up days to get rid of mosquito breeding sites. Community outreach involves presenting messages in person, in addition to media and educational materials, and involving citizens in prevention activities. Hearing the message of personal prevention from community leaders can validate the importance of the disease. Health promotion events and activities reinforce the importance of prevention in a community setting.

Partnership with Media and the Community

It can be beneficial to cultivate relationships with the media (e.g., radio, TV, newspaper, web-based news outlets) prior to an outbreak. Obtain media training for at least one member of your staff and designate that individual as the organization's spokesperson. Develop clear press releases and an efficient system to answer press inquiries. Many communities have heard similar prevention messages repeated for several years. Securing the public's attention when risk levels increase can be a challenge. Evaluate and update mosquito bite prevention messages annually, and test new messages with different population segments to evaluate effectiveness. Develop partnerships with agencies/organizations that have relationships with populations at higher risk (such as persons over 50 years of age) or are recognized as community leaders (e.g., churches, service groups). Working through sources trusted by the priority

audience can heighten the credibility of and attention to messages. Partnerships with businesses that sell materials to fix or install window screens or that sell insect repellent may be useful in some settings (e.g., local hardware stores, grocery stores).

Social Media

Social media can be an inexpensive and rapid method for disseminating information to the community. Outreach can be conducted using Twitter, Facebook, YouTube, blogs, and other websites that may reach constituents less connected to more traditional media sources. Using images or videos in your posts make them more attention grabbing. It is also best practice to include a call-to-action people can take. Provide links that direct users to webpages or other resources with more complete information.

Online Resources

The Internet has become a primary source of health information for many Americans. Encourage constituents to seek advice from credible sources. Make sure local public health agency websites are clear, accurate, and up to date. Useful information is available from a number of resources:

- The CDC web pages are updated frequently to reflect new findings and recommendations. Materials on the CDC web site are in the public domain and serve as a resource for state and local health departments and other organizations.
- CDC staff can provide technical assistance in the development of audience research and strategies for public education and community outreach. Contact CDC/Division of Vector-Borne Diseases' health communications staff in Fort Collins, CO at 970-221-6400.
- The U.S. Environmental Protection Agency (EPA) is the government's regulatory agency for insecticide and insect repellent use, safety, and effectiveness. Information about mosquito control insecticides and repellents is available at https://www.epa.gov/mosquitocontrol . These include guidance for using insect repellents safely (https://www.epa.gov/pesticides/insect/safe.htm) and a search tool to assist in finding an insect repellent that is right for you (http://cfpub.epa.gov/oppref/insect/#searchform) which allows the user to examine the protection time afforded by registered insect repellents containing various concentrations of the active ingredients.

There are a number of non-governmental organizations that have developed useful tools and information that can be adapted for local needs. Examples include: the American Mosquito Control Association (https://www.mosquito.org/default.aspx) and the National Pesticide Information Center (NPIC) (npic.orst.edu).

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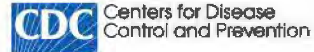

Prevention and Control: Integrated Vector Management

Integrated Vector Management

Prevention and control of arboviral diseases is accomplished most effectively through a comprehensive, integrated vector management (IVM) program applying the principles of integrated pest management. IVM is based on an understanding of the underlying biology of the arbovirus transmission system and utilizes regular monitoring of vector mosquito populations and arboviral activity levels to determine if, when, and where interventions are needed to keep mosquito numbers below levels which produce risk of human disease, and to respond appropriately to reduce risk when it exceeds acceptable levels.

Operationally, IVM is anchored by a monitoring program providing data that describe:

- Conditions and habitats that produce vector mosquitoes.
- Abundance of those mosquitoes over the course of a season.
- Arboviral transmission activity levels expressed as infection rate in mosquito vectors.
- Parameters that influence local mosquito populations and virus transmission.

These data inform decisions about implementing mosquito control activities appropriate to the situation, such as:

- Source reduction through habitat modification.
- Larval mosquito control using the appropriate methods for the habitat.
- Adult mosquito control using pesticides applied from trucks or aircraft when established thresholds have been exceeded.
- Community education efforts related to risk levels and intervention activities.

Monitoring also provides quality control for the program, allowing evaluation of the effectiveness of larval and adult control efforts, and causes of control failures (e.g., undetected larval sources, pesticide resistance, equipment failure).

Mosquito Control Activities

Guided by the surveillance elements of the program, integrated efforts to control mosquitoes are implemented to maintain vector populations below thresholds that would facilitate virus amplification and increase human risk (Table 1) (Nasci and Mutebi, 2019).

Larval Mosquito Control

The objective of the larval mosquito control component of an IVM program is to manage mosquito populations before they emerge as adults. This can be an efficient method if the mosquito breeding sites are accessible. However, larval control may not attain the levels of mosquito population reduction needed to maintain risk at low levels and must be accompanied by measures to control the adult mosquito populations as well. In outbreak situations, larval control complements adult mosquito control measures by preventing new vector mosquitoes from being produced. However, larval control alone is not able to stop outbreaks once virus amplification has reached levels causing human infections.

Numerous methods are available for controlling larval mosquitoes. Source reduction is the elimination or removal of habitats that produce mosquitoes. This can range from draining roadside ditches to properly disposing of discarded tires and other trash containers. Only through a thorough surveillance program will mosquito sources be identified and appropriately removed. In order to effectively control vector mosquito populations through source reduction, all sites capable of producing vector mosquitoes must be identified and routinely inspected for the presence of mosquito larvae or pupae. This is difficult to accomplish with the vector species *Cx. quinquefasciatus* and *Cx. pipiens* that readily utilize cryptic sites such as storm drainage systems, grey water storage cisterns, and storm water runoff impoundments. Vacant housing with unmaintained swimming pools, ponds and similar water features are difficult to identify and contribute a significant number of adult mosquitoes to local populations.

To manage mosquitoes produced in habitats that are not conducive to source reduction, pesticides registered by EPA for larval mosquito control are applied when larvae are detected. No single larvicide product will work effectively in every habitat where vectors are found. Information about pesticides used for larval mosquito control is available from the U.S. EPA (https://www.epa.gov/mosquitocontrol/controlling-mosquitoes-larval-stage). Pesticides should always be used according to their label instructions by field staff trained to identify larval production sites and safely implement the appropriate management tools for that site.

Adult Mosquito Control

Source reduction and larvicide treatments may be inadequate to maintain vector populations at levels sufficiently low to limit virus amplification. The objective of the adult mosquito control component of an IVM program is to complement the larval management program by reducing the abundance of adult mosquitoes in an area, thereby reducing the number of eggs laid in breeding sites. Adult mosquito control is also intended to reduce the abundance of biting, infected adult mosquitoes in order to prevent them from transmitting virus to humans and to break the mosquito-bird transmission cycle.

In situations where vector abundance is increasing above acceptable levels, targeted adulticide applications using pesticides registered by EPA for this purpose can assist in maintaining vector abundance below threshold levels. More detailed information about pesticides used for adult mosquito control is available from the U.S. EPA (https://www.epa.gov/mosquitocontrol/controlling-adult-mosquitoes).

Pesticides for adult mosquito control can be applied from hand-held application devices, from trucks or aircrafts. Hand-held or truck-based applications are useful to manage relatively small areas but are limited in their capacity to treat large areas quickly during an outbreak. Gaps in coverage may occur during truck-based applications due to limitations of the road infrastructure. Aerial application of mosquito control adulticides is used when large areas must be treated quickly. Aerial spraying can be particularly valuable to control *Cx. quinquefasciatus* or *Cx. pipiens* which require multiple, closely timed treatments. Both truck and aerially-applied pesticides are applied using ultra-low-volume (ULV) technology in which a very small volume of pesticide is applied per acre in an aerosol of minute droplets designed to contain sufficient pesticide to kill mosquitoes that are contacted by the droplets. Information describing ULV spray technology and the factors affecting effectiveness of ground and aerially applied ULV pesticides is reviewed in Mount et al. 1996, Mount 1998, and Bonds 2012.

Vector Management in Public Health Emergencies

Intensive early season adult mosquito control efforts can decrease viral transmission activity and result in reduced human risk (Lothrop et al. 2008). However, depending on local conditions, proactive vector management may not maintain mosquito populations at levels sufficiently low to avoid development of outbreaks. As evidence of sustained or intensified virus transmission in a region increases, emergency vector control efforts to reduce the abundance of infected, biting adult mosquitoes must be implemented. This is particularly important in areas where vector surveillance indicates that infection rates in mosquitoes are continually increasing or being sustained at high levels and evidence of infection found in other species (e.g., human or non-human mammal cases). Delaying adulticide applications until numerous human cases occur negates the value and purpose of the surveillance system. Timely application of adulticides interrupts arboviral transmission and prevents human cases (Carney et al. 2008).

Safety and Quality of Vector Control Pesticides and Practices

Insecticides to control larval and adult mosquitoes are registered specifically for that use by the U.S. Environmental Protection Agency (EPA). Instructions provided on the product labels prescribe the required application and use parameters and must be carefully followed. Properly applied, these products do not negatively affect human health or the environment. In persons living in treated areas, ULV application of mosquito control adulticides does not produce any detectable biological changes indicating exposure (Currier et al. 2005, Duprey et al. 2008) or increase asthma or other adverse health events (Karpati et al. 2004). The risks from arboviruses demonstrably exceed the risks from mosquito control practices (Davis and Peterson 2008, Macedo et al. 2010, Peterson et al. 2006).

Legal Action to Achieve Access or Control

Individually owned private properties may be major sources of mosquito production. Examples include accumulations of discarded tires or other trash, neglected swimming pools, and similar water features that become stagnant and produce mosquitoes. Local public health statutes or public nuisance regulations may be employed to gain access for surveillance and control, or to require the property owner to mitigate the problem. Executing such legal actions may be a prolonged process during which adult mosquitoes are continuously produced. Proactive communication with residents and public education programs may alleviate the need to use legal actions. However, legal efforts may be required to eliminate persistent mosquito production sites.

Quality of Control

Pesticide products and application procedures (for both larval and adult control) must periodically be evaluated to ensure an effective rate of application is being used and that the desired degree of control is obtained. Application procedures should be evaluated regularly (minimally once each season) to assure equipment is functioning properly to deliver the correct dosages and droplet parameters and to determine appropriate label rates to use locally. Finally, mosquito populations should routinely be evaluated to ensure insecticide resistance is not emerging.

Records

Surveillance data describing vector sources, abundance and infection rates, records of control efforts (e.g., source reduction, larvicide applications, adulticide applications), and quality control data must be maintained and used to evaluate IVM needs and performance. Long-term data are essential to track trends and to evaluate levels of risk.

Insecticide Resistance Management

For vector control to be effective, mosquitoes must be susceptible to the insecticide selected for use. In order to delay or prevent the development of insecticide resistance in vector populations, IVM programs should include a resistance management component (Florida Coordinating Council on Mosquito Control 1998). This should include routine monitoring of the status of resistance in the target populations to:

- Provide baseline data for program planning and pesticide selection before the start of control operations.
- Detect resistance at an early stage so that timely management can be implemented.
- Continuously monitor the effect of control strategies on insecticide resistance, and determine potential causes for control failures, should they occur.

Insecticide resistance may be monitored using bioassays in larvae or adult mosquitoes (Brogden and McAllister 1998). The CDC bottle bioassay is a simple, rapid, and economical tool to detect insecticide resistance by determining the time taken for a pesticide active ingredient to kill mosquito vectors. The results can help guide the choice of insecticide used for spraying. The CDC bottle bioassay can be used as part of a broader insecticide resistance monitoring program, which may include field cage tests and biochemical and molecular methods. A practical laboratory manual for the CDC bottle bioassay is available online (https://www.cdc.gov/mosquitoes/mosquito-control/professionals/cdc-bottle-bioassay.html). For additional information contact CDC at USBottleAssayKit@cdc.gov.

The IVM program should include options for managing resistance that are appropriate for the local conditions. The techniques regularly used include the following:

- Management by moderation. Preventing onset of resistance by reducing overall chemical use or persistence:
  - Using dosages no lower than the lowest label rate to avoid genetic selection.

- Using chemicals of short environmental persistence and avoiding slow-release formulations that increase selection for resistance.
- Avoiding use of the same class of insecticide to control adult and immature stages.
- Applying locally: many districts treat only hot spots and use area-wide treatments only during public health alerts or outbreaks.
- Using less frequent applications; leaving generations, population segments, or areas untreated (when appropriate).
- Establishing higher thresholds for mosquito mitigation with insecticides, except during public health alerts or outbreaks.

- Management by continued suppression. This strategy is used in regions of high value or persistent high risk (e.g., heavily populated regions or locations with recurring WNV outbreaks) where mosquitoes must be kept at very low densities. It involves the application of dosages within label rates but sufficiently high to be lethal to heterozygous individuals that are partially resistant. If the heterozygous individuals are killed, resistance will be slow to emerge. This method should not be used if any significant portion of the population in question is fully resistant. Another approach more commonly used is the addition of synergists that inhibit existing detoxification enzymes and thus eliminate the competitive advantage of these individuals. Commonly, the synergist of choice in mosquito control is piperonyl butoxide (PBO).
- Management by multiple attack. This strategy involves the use of insecticides with different modes of action in mixtures or in rotations. There are economic limitations associated with this approach (e.g., costs and logistics of switching or storing chemicals), and critical variables in addition to the pesticide mode of action that must be taken into consideration (i.e., mode of resistance inheritance, frequency of mutations, population dynamics of the target species, availability of refuges, and migration). Programs should evaluate resistance patterns routinely and the need for rotating insecticides at annual or longer intervals.

Continuing Education

Continuing education for operational vector control workers is required to instill or refresh knowledge related to practical mosquito control. Training focusses on safety, applied technology, and requirements for the regulated certification program mandated by most states. Training should also include information on the identification of mosquito species, their behavior, ecology, and appropriate methods of control.

Guidelines for a Phased Response

The objective of a phased response to WNV surveillance data is to implement public health interventions appropriate to the level of WNV risk in a community (Table 1). A surveillance program adequate to monitor WNV activity levels associated with human risk must be in place in order to provide detection of epizootic transmission in advance of human disease outbreaks. The surveillance programs and environmental surveillance indicators described above demonstrate that enzootic/epizootic WNV transmission can be detected several weeks before the onset of human disease, allowing for implementation of effective interventions (Bolling *et al.* 2009, Jones *et al.* 2011, Mostashari et al. 2003, Unlu et al. 2009).

All communities should prepare for WNV activity. For reasons that are not well understood, some regions are at risk of higher levels of WNV transmission and epidemics than others (CDC 2010), but there is evidence of WNV presence and the risk of human disease and outbreaks in most counties in the contiguous 48 states. The ability to develop a useful phased response depends upon the existence of some form of WNV monitoring in the community to provide the information needed to gauge risk levels. Measures of the intensity of WNV epizootic transmission in a region, preferably from environmental surveillance indicators, should be considered when determining the level of the public health response. As noted previously, human case reports lag weeks behind human infection events and are poor indicators of current risk levels. Effective public health actions depend on interpreting the best available surveillance data and initiating prompt and aggressive intervention when necessary.

Recommendations for a phased response to WNV surveillance data

Risk category	Probability of human outbreak	Definition	Recommended activities and responses

Risk category	Probability of human outbreak	Definition	Recommended activities and responses
0	None	• No adult mosquito biting activity (vector species).	• Develop and review WNV response plan. • Review mosquito control program. • Maintain source reduction projects. • Secure surveillance and control resources necessary to enable emergency response. • Review and update community outreach and public education programs.
1	Low	• Biting adult mosquitoes active (vector species) -or- • Epizootic activity expected based on onset of transmission in prior years -or- • Limited or sporadic epizootic activity in birds or mosquitoes.	• Response as in category 0, plus: • Conduct IVM program to monitor and reduce vector mosquito abundance. • Conduct environmental surveillance to monitor virus activity (mosquitoes, sentinel chickens, avian mortality, etc.). • Initiate community outreach and public education programs focused on personal protection and residential source reduction.
2	High	• Sustained transmission activity in mosquitoes or birds -or- • Horse cases reported -or- • Human case or viremic blood donor reported.	• Response as in category 1 plus: • Intensify and expand adult mosquito control in areas using ground and/or aerial applications where surveillance indicates human risk. • Intensify visible activities in community to increase attention to WNV transmission risk and personal protection measures. • Work with collaborators to address high-risk populations. • Intensify and expand surveillance for human cases.

Risk category	Probability of human outbreak	Definition	Recommended activities and responses
3	Outbreak in progress	• Conditions favor continued transmission to humans (i.e., persistent high infection rate in mosquitoes, continued avian mortality, seasonal mosquito population decreases not anticipated for weeks) -or- • Multiple confirmed human cases or viremic blood donors.	• Response as in category 2 plus: • Intensify emergency adult mosquito control program repeating applications as necessary to achieve adequate control. • Monitor effectiveness of vector control efforts. • Emphasize urgency of personal protection, including use of repellents, through community leaders and media.

References

Bonds JA. 2012. Ultra-low-volume space sprays in mosquito control: a critical review. *Med Vet Entomol.* 26(2):121-30.

Brogdon WG, McAllister JC. 1998. Insecticide resistance and vector control. *Emerging Infectious Diseases* 4:605-613.

Carney RM, Husted S, Jean S, Glaser C, Kramer V. 2008. Efficacy of aerial spraying of mosquito adulticide in reducing incidence of West Nile virus, California. *Emerg Infect Dis.* 14:747-754. doi: 10.3201/eid1405.071347

Currier M, McNeill, M, Campbell D, Newton N, Marr JS Perry E, Berg SW, Barr DB, Luber GE, Kieszak MA, Rogers HS, Backer LC Belson MG Bubin C Azziz-Baumgartner E, Duprey ZH. 2005. Human exposure to mosquito-control pesticides-Mississippi, North Carolina, and Virginia, 2002 and 2003. *MMWR.* 54:529-532.

Davis RS, Peterson RK. 2008. Effects of single and multiple applications of mosquito insecticides on nontarget arthropods. *J Am Mosq Control Assoc.* 24-270-280. Doi:10.2987/5654.1 50

Duprey Z, Rivers S, Luber G, Becker A, Blackmore C, Barr D, Weerasekera G, Kieszak S, Flanders WD, Rubin C. 2008. Community aerial mosquito control and naled exposure. *J Am Mosq Control Assoc.* 24:42-46. Doi:10.2987/5559.1

Florida Coordinating Committee Mosquito Control. 1998. Florida mosquito control: the state mission as defined by mosquito controllers, regulators, and environmental managers. Gainesville, FL: University of Florida. Available from: URL: http://fmel.ifas.ufl.edu/white_paper/FWP09.pdf. Accessed 5/7/2013external icon

Karpati AM, Perrin MC, Matte T, Leighton J, Schwartz J, Barr RG. 2004. Pesticide spraying for West Nile virus control and emergency department asthma visits in New York City, 2000. *Environ Health Perspect.* 112(11):1183-7.

Macedo, PA, Schleier, III JJ, Reed M, Kelley K, Goodman GW, Brown DA and Peterson RKD. 2010. Evaluation of efficacy and human health risk of aerial ultra-low volume applications of pyrethrins and piperonyl butoxide for adult mosquito management in response to West Nile virus activity in Sacramento County, California. *J Am Mosq Control Assoc.* 26-57-66. Doi: 10.2987/09-5961.1

Lothrop HD, Lothrop BB, Gomsi DE and Reisen WK. 2008. Intensive early season adulticide applications decrease arbovirus transmission throughout the Coachella Valley, Riverside County, California. *Vector Borne and Zoonotic Dis.* 8:475-490. doi: 10.1089/vbz.2007.0238

Mount GA. 1998. A critical review of ultralow-volume aerosols of insecticide applied with vehicle-mounted generators for adult mosquito control. *J Am Mosq Control Assoc.* 14(3):305-34.

Mount GA, Biery TL, Haile DG. 1996. A review of ultralow-volume aerial sprays of insecticide for mosquito control. *J Am Mosq Control Assoc.* 12(4):601-18.

Nasci RS, Mutebi JP. 2019. Reducing West Nile virus through vector management. *J Med Entomol. 56(6):1516-1521.*

Peterson RKD, Macedo PA, Davis RS. 2006. A human-health risk assessment for West Nile virus and insecticides used in mosquito management. *Environ Health Perspect.* 114-366-372. Doi:10.1289/ehp.8667

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Peterson RKD, Macedo PA, Davis RS. 2006. A human-health risk assessment for West Nile virus and insecticides used in mosquito management. *Environ Health Perspect.* 114-366-372. Doi:10.1289/ehp.8667

Bolling BG, Barker CM, Moore CG, Pape WJ, Eisen L. 2009. Seasonal patterns for entomological measures of risk for exposure to *Culex* vectors and West Nile virus in relation to human disease cases in northeastern Colorado. *J Med Entomol.* 46(6):1519-31. Doi:10.1111/j.1365-2915.2012.01014.x.Epub2012 Apr 10

McDonald et al. 2010. Surveillance for human West Nile virus disease – United States, 1999-2008. *MMWR.* 59(No SS-2):1-17.

Jones RC, Weaver KN, Smith S, Blanco C, Flores C, Gibbs, K, Markowski D, Mutebi JP. 2011. Use of the Vector Index and geographic information system to prospectively inform West Nile virus interventions. *J Am Mosq Control Assoc.* 27(3): 315-19. Doi: 10.2987/10-6098.1

Mostashari F, Kulldorff M, Hartman JJ, Miller JR, Kulasekera V. 2003. Dead bird clusters as an early warning system for West Nile virus activity. *Emerg Infect Dis.* 9:641-646. Doi: 10.3201/eid0906.020794

Unlu I, Roy AF, Yates M, Garrett D, Bell H, Harden T, Foil LD. 2009. Evaluation of surveillance methods for detection of West Nile virus activity in East Baton Rouge Parish, Louisiana, 2004-2006. *J Am Mosq Control Assoc.* 25(2):126-33. Doi:10.2987/08-5713.1

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Appendix 2: Interim Guidance for States Conducting Avian Mortality Surveillance for West Nile Virus (WNV) or Highly Pathogenic H5N1 Avian Influenza Virus

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