A Model Surveillance Program for Vectorborne Diseases in California, 1999-2000

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ABSTRACT: This report summarizes research during year 3 of a 5-year project to improve the efficiency of the California Vectorborne Disease Surveillance Program. Complete results are contained among the publications cited below. Enhanced surveillance for arboviral encephalitis in humans continued, but failed to detect any new cases. A study of grain-baited traps for surveillance of arboviral activity in wild birds concluded that wild birds are not suitable for rural surveillance because of lack of sensitivity and difficulties in interpretation of serological results. Further improvements were made to the surveillance website, including incorporation of automation for updating of databases and direct control of databases by testing laboratories. Future prospects for surveillance for vector-borne diseases in California are discussed.

INTRODUCTION

This report summarizes research during year 3 of a 5-year project to improve the efficiency of the California Vectorborne Disease Surveillance Program and is based on presentations at a surveillance symposium held at the Mosquito and Vector Control Association of California (MVCAC) annual conference in January, 2000 in Sacramento. Previous reports summarized the 5 interrelated objectives of the program. Included in this report are (1) the results of enhanced human surveillance for arbovirus infections in California, (2) an evaluation of the ability of grain-baited traps to focus on indicator bird species for surveillance, (3) the results of a mark-release-recapture experiment conducted in the San Joaquin River delta, (4) the development of new methods for the monitoring of insecticide resistance, (5) improvements made to the electronic reporting of results of arbovirus surveillance, and (6) an examination of the future of surveillance in California. Many people participated in the research presented herein. The individuals who made presentations at the symposium are listed as authors, and other participants are acknowledged at the end of the article.

1. Enhanced Human Arbovirus Surveillance

In 1999, attempts were made to detect acute human infections caused by St. Louis encephalitis (SLE) and western equine encephalomyelitis (WEE) viruses. One study, supported by special funds from MVCAC, focused on health care providers in Riverside and Imperial Counties. Results also were presented from an ongoing encephalitis project in California conducted by the California Department of Health Services (DHS), designated the California Encephalitis Project.

SLE and WEE in Imperial and Riverside Counties

Human case detection

A goal of this study was to increase physician awareness of the potential for mosquito-borne viral infections in humans, and to encourage the submission of specimens from individuals presenting symptoms indicative of possible arbovirus infections. In addition, a background serosurvey based on convenience samples gathered data on previous infections. For case detection, physicians were alerted via a letter with an attached fact sheet on SLE and WEE infections, a case history form, and instructions for submitting specimens. Posters were placed in emergency rooms and clinics, and presentations were made to physicians.

Specimens from a total of 15 cases were submitted. Symptoms included febrile headache (11 cases), encephalitis (2) and vertigo (1). Eight of the specimens were from Riverside County, 7 were from Imperial County. Only one acute phase specimen showed IgG antibodies; none showed IgM antibodies.
No acute cases of St. Louis encephalitis or western equine encephalomyelitis were detected via this project. Unfortunately, physician compliance was low, resulting in few specimens for testing.

Serosurvey

Blind samples of bloods drawn for other medical purposes were obtained from 4 different medical facilities (3 hospitals and 1 plasma center) and tested by ELISA for IgG antibodies to SLE and WEE. A total of 729 bloods were collected from individuals ranging from 1 to 99 years of age (mean age 45). Males represented 56% of the specimens, females 44%.

Of the 729 samples tested, 116 showed IgG antibodies to SLE, 11 to WEE. In one specimen, SLE IgM was detected. As with all of these specimens, there is no associated clinical information. A follow-up study is in progress.

There was a high level of seropositivity to SLE among the 729 specimens, but there are problems with interpretation because dengue (DEN), West Nile (WN) and yellow fever (YF) viruses all cross-react with SLE. Cross neutralization tests on the IgM positive SLE will be conducted for DEN, SLE, YF and possibly WN at the UC Davis Arbovirus Research Unit (ARU).

California Encephalitis Project

The goal of this long-term project is to establish the causes of encephalitis detected in California. The criteria for inclusion in this study are hospitalization with encephalopathy; depressed or altered level of consciousness for >24 h; lethargy or change in personality, and one or more of the following: fever, seizures, worsening neurologic findings, CSF pleocytosis, EEG finding consistent with encephalitis, or abnormal neuroimaging. Case patients must be older than 6 months of age.

No cases of western equine encephalomyelitis were confirmed. Of 190 cases examined to date, 8 were positive for IgG antibodies. There have been no IgM positive specimens.

All cases with rural exposure or mosquito exposure tested were sent to the UC Davis ARU for further testing. Of 37 specimens tested, all were negative for California encephalitis virus, SLE and WEE.

2. Enzootic surveillance: ability of grain-baited traps to focus on indicator bird species

Introduction

The ARU is studying the role of wild birds in the maintenance and amplification of WEE and SLE in wetland habitats of California. These studies currently indicate that avian seroprevalence rates generally remain low and that most infections occur in relatively few species (Reisen et al. 2000); mostly house finches, house sparrows, California and Gambel's quail, and mourning and common ground doves that are sampled effectively by both mist nets and grain baited traps. The purpose of our research during 1999 was to convert our labor-intensive, mist net sampling into an efficient trapping program that mosquito control districts could use to augment encephalitis virus surveillance in rural areas. A similar transition in sampling was accomplished previously in suburban Orange County (Gruwell et al. 1988; McLean et al. 1988). Additional observations on antibody persistence were included because these data relate strongly to the interpretation of seroprevalence data, especially during the critical spring period.

Specifically, our objectives were to:

3. Describe field and experimental data on antibody persistence.

Virus Activity

Field research focused on the southern Coachella Valley near the Salton Sea in Riverside County and the Bakersfield area in the southern San Joaquin Valley in Kern County. One sentinel chicken flock, 1-3 grain-baited ground traps, a modified crow trap, and 1-3 dry ice-baited CDC style mosquito traps were positioned at each site. Sampling in Coachella Valley included 8 sites and was extended into the upper valley in an attempt to trap more house finches and mourning doves. In Kern County, sampling was limited to the Kern River and Tracy Ranch to focus on California quail and house finch populations, respectively. Sentinel chickens were bled from the comb, whereas wild birds were bled by jugular puncture. Birds were screened for antibody by appropriate enzyme immunoassays (EIA). Seropositive chickens were confirmed by an indirect fluorescent antibody test, whereas seropositive wild birds were confirmed by a plaque reduction neutralization test (PRNT). Mosquitoes were identified to species, enumerated, and Cx. tarsalis were pooled for virus isolation using Vero cell culture followed by an in situ EIA.

Detection of virus activity was low in Coachella Valley and absent from the southern San Joaquin Valley during 1999 (Table 1). In the Coachella Valley, WEE infection was detected in a single sentinel chicken.

Summary

Overall, our data indicated that:
1. Grain-baited traps focused sampling on target bird species, but failed to collect sufficient numbers of house finches and mourning doves for effective surveillance.
2. Catch in traps was temporally variable and frequently hampered by predators.
3. Sensitivity to presence of arboviruses could not be compared effectively during 1999, because WEE and SLE were not sufficiently active in our study areas.

4. WEE antibody persisted over winter in recaptured quail, but titers decreased.

5. WEE antibody persisted over the winter in experimentally infected house finches and protected against reinfection, whereas SLE antibody decayed rapidly and some birds produced viremia upon rechallenge.

6. Based on the current and previous observations, we concluded that a wild bird surveillance program was not suitable for rural surveillance by mosquito and vector control districts, because:


   b. Interpretation. Variable rates of antibody decay, possible reinfection, and low antibody titers made it difficult to separate recent from previous infections testing for IgG. The only data suitable to detect early season virus activity was infection in hatching-year or recaptured birds. However, hatching-year birds were infected mostly during midsummer, and after virus amplification was detected by seroconversions among sentinel chickens or virus was isolated from mosquitoes. The numbers of birds that were collected antibody negative, banded and then recaptured antibody positive, was too low for sensitive surveillance.

   c. Consistent data. We had problems with trap efficiency changing over time based on nesting behavior, presence of alternative food and predators.

3. Surveillance for Pesticide Resistance in California

   The concept of vectorborne disease surveillance was broadened to include surveillance for pesticide resistance in response to guidelines of the Epidemic Arbovirus Response Working Group, a joint effort of MVCA, DHS, and the University of California. The rationale for the inclusion of pesticide surveillance was the realization that presence of resistance in vector mosquitoes is dynamic, and must be studied in advance of potential outbreaks and for management of pesticide resistance.

First reports of apparent methoprene control failures against *Aedes nigromaculis* were noted in a pasture west of Fresno in September 1998. Methoprene failures had spread to an additional 10 pastures in Fresno County during the summer of 1999. In some of these pastures, methoprene, a juvenile hormone analog, had been used for at least 20 years as the primary insecticide to control this mosquito. Field trials, based on pupal counts and different methoprene application rates, showed that in some pastures, either no control, or only low levels of control, were achieved with Alotisid Liquid Larvicide (ALL®) and Alotisid® XR-G and 52-99% control with Alotisid® Pellets (Cornel et al. 2000).

A methoprene bioassay for *Ae. nigromaculis* was developed. Preliminary results indicated that there was a methoprene tolerance difference of 50- to 100-fold The purity rate of unmarked mosquitoes captured during the same period was slightly higher but did not exceed 28%. This low rate of feeding success suggests a lower risk of virus transmission at the time and place the study was conducted. Estimates of daily survivorship varied among release groups but were consistent with previous studies and suggested that some individuals could survive long enough to acquire and transmit encephalitis viruses. Population density estimates, calculated by the modified Lincoln Index method, averaged approximately 18,800/km². We concluded that mosquitoes produced at sources in the delta have the potential to rapidly impact nearby populated areas, and that successful control of *Cx. tarsalis* in this area, and reduction of disease risk, will require inter-agency cooperation, including sharing of pertinent data on a
between a methoprene-naïve population and populations from pastures where methoprene had been used consistently for 6 to 20 years.

In preparation for bottle bioassay surveillance of Cx. pipiens complex and Cx. tarsalis mosquitoes, standard susceptible colonies of these mosquitoes were established. These colonies are susceptible to all of the chemical larvicides and adulticides registered for mosquito control in California.

4. Mark-release-recapture studies of Culex tarsalis in the San Joaquin delta
The objectives of this study were to investigate the flight range and distance, population dynamics and population density of the encephalitis mosquito, Cx. tarsalis, on islands in the San Joaquin River delta along the border between Contra Costa and San Joaquin Counties.

Adult female Cx. tarsalis were collected for release in dry ice-baited EVS traps at 2 locations on 3 consecutive nights in August 1999. Each of the 3 collections were transported to the release point at the southern end of Madelaine Island (San Joaquin County) on the evening after capture, were marked with 3 different colors of fluorescent powder (pink, green and blue on nights 1, 2, and 3 respectively) and released at dusk. Of a total of 57,247 released, 305 marked individuals were recaptured during the following 9 evenings in a total of 60 EVS (battery-operated CO₂-baited) traps located in roughly concentric rings within a 7 km radius from the release point. The predominant flight direction was west (upwind). The rate of dispersal was greater than had been anticipated based on previous studies, with recaptured mosquitoes moving an average of 3.6 km per night and a maximum of 5 km in a single night, easily crossing among islands. We dissected the ovaries of the majority of marked mosquitoes recaptured and found that less than 10% of individuals released successfully fled and reproduced during the study period.

5. Analysis, prediction and reporting
A surveillance website is located at http://mosqnet.ucdavis.edu. Further improvements were made in 1999 in the electronic reporting of arbovirus surveillance information. A new concept for maintenance of database tables was incorporated in which the testing laboratory involved becomes the "owner" of the data, which are maintained on a central server. Testing laboratories are responsible for periodic updates to the data. Progress has been made on connecting laboratories directly to the server, but some problems remain. Much of the drudgery of updating maps and data surveillance summaries has been eliminated through installation of programs that automatically update the website once the data tables have been updated.

Financial support has been requested from the Centers for Disease Control and Prevention (CDC) to install a further extension of the system whereby mosquito agencies throughout California will have full access to the database. By using specially designed client software, a full range of analysis options, including mapping, graphing and various types of reporting will be available. Under the present system, analysis of historical data is difficult; under the new concept, users could select any time frame desired. The implementation of this concept is being coordinated with Advance Computer Resources, Inc. A demonstration mapping server is planned for 2000.

6. The future of Surveillance for Arbovirus Diseases
The arbovirus surveillance system in California has 2 goals. First, to predict the threat of virus transmission to people, in other words, to serve as an early warning system. Second, to document local transmission; to confirm that a given virus is present and is being transmitted. Monitoring of enzootic virus activity is the primary method used currently in California to estimate risk of human infection. However, the relationship between enzootic transmission of arboviruses and human infections is unclear. Consequently, this complicates predicting human infection based on enzootic transmission even though past studies in California have shown that critical decisions regarding risk of human infection can be based on the density of Cx. tarsalis (Reeves 1971).

Facts about arboviral encephalitis
The arboviral encephalitides continue to constitute a significant public health burden. Although the number of confirmed human cases of arboviral encephalitis has declined annually in recent years to just a few in California, the incidence nationwide continues to be 150–3,000 per year. Total cost association with these cases is considerable, and is currently estimated to be $1.50 million per year, including costs of vector control and surveillance activities. Cost of individual cases is also substantial. It has been suggested that costs for an individual case of eastern equine encephalomyelitis range from $15,000 to $50,000. The disease in the United States is often fatal, human disease, had gone from being absent to occurring in much of tropical America. The devastating pattern reported in the 1950s of dengue emerging as a major public health threat was repeating itself in tropical America, in part because efforts to control Ae. aegypti either ceased or were significantly reduced.

The recent appearance of West Nile virus (WN) in the northeastern USA similarly points out that we should not limit our vigilance only to viruses that we know are present in California. We no longer can limit our attention to activities within the boundaries of the state of California. We must prepare to respond to vector-borne pathogens that may be introduced into California accidentally or on purpose (bioterrorism). This will require expertise and research on vector-borne pathogens that historically have not been detected in the state.

The WN outbreak in New York, which presumably resulted from the introduction of virus from the Old World, resulted in over 60 human cases and 7 deaths. Virus was isolated from 18 species of birds, mostly crows, within 50 miles of New York City. Virus was isolated from a dead crow in Baltimore, about 250 mi from New York City. Serosurveys produced evidence of human WNV infection in at least 15 states.
$21,000 for transiently infected individuals to $3 million for severely infected people (Villari et al. 1995).

The real threat to humans from arboviral diseases

The annual number of cases of western equine encephalomyelitis and St. Louis encephalitis occurring in California recently may suggest to some that the risk of human infection by these agents is virtually nonexistent (Reeves 1990). However, before concluding that there is little risk of future outbreaks of arboviral diseases, one should look at some disturbing trends for arboviral diseases worldwide. For example, the history of dengue in the Americas points out that we must not become complacent about sustaining California’s outstanding arbovirus surveillance program.

In 1970, due to a Pan American Health Organization supported hemisphere-wide eradication campaign, the geographic range of Aedes aegypti, the primary vector of dengue virus, was restricted to just a few isolated areas in the Americas: the southeastern USA, the Caribbean region, and northeastern South America. By 1997, following relaxation of the eradication program, it has spread to most of Mexico, all of Central America, and parts of nearly every South American country. During roughly this same time period, dengue hemorrhagic fever, the cause of severe, respectively. Anopheline vectors of malarial parasites occur in California, the parasites are being introduced into the state, and the threat of autochthonous transmission exists.

MMWR reports on imported dengue similarly support the notion that programs for detecting and responding to imported vector-borne pathogens need to be in place in California. In 1995, 86% of 441 people with dengue-like illness from 31 states and the District of Columbia were reported as having dengue. Of those people with confirmed dengue, 83 had recently traveled to the Caribbean (48), Mexico and Central America (24), Asia (5), and Africa (6). In 1996, 24% of 179 people with dengue-like symptoms from 32 states and DC were diagnosed as having dengue. Thirty-seven people had recent travel histories to the Caribbean (19), Asia (11), Africa (3), Pacific Islands (2) and Central and South America (2). Results from a study limited to the state of Florida during 1997-1998 indicate that of 83 people suspected of having dengue, the disease was confirmed for 36. All reported travel within 10 days of onset of illness to Haiti, Puerto Rico, Colombia, Venezuela, Barbados, Nicaragua or Thailand.

Malaria and dengue are reportable diseases; they are disease agents that are accounted for by state and federal officials. But there is a wide variety of other vector-borne pathogens that could also be introduced into California and could go undetected. On a worldwide basis, there are hundreds of arboviruses that may pose a threat from introduction. Examples among the arboviral encephalitides include West Nile encephalitis, Venezuelan equine encephalomyelitis, tick-borne encephalitis, Japanese encephalitis, and Murray Valley encephalitis, tick-borne encephalitis, and Murray Valley encephalitis.

Emerging diseases

In recent years there has been a fascination in the popular press, films, and in scientific circles with the so-called emerging diseases. Morse and Schluederberg (1990) provide a working definition for these kinds of pathogens. They explain that emerging diseases are caused by pathogens that either have newly appeared in a population or are rapidly expanding their range, with a corresponding increase in disease cases.

One could ask, does the introduction of vector-borne pathogens constitute a public hazard to California? We present 2 examples that indicate that introduced pathogens do constitute a threat for the citizens in our state. In fact, the WN epidemic in New York City points out that accidental or purposeful introduction of pathogens will happen. Therefore, vector control and public health officials need to be prepared to detect and respond to these situations when they occur.

According to Morbidity and Mortality Weekly Reports (MMWR), published by the CDC, each year from 1966 to 1999 a few hundred to over 3,000 cases of imported malaria were documented in the United States. In California alone, during 1998 and 1999, there were 217 and 208 reported imported malaria cases.

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Plans to provide adequate protection to citizens of California against the risk of infection with emerging arthropod-borne infectious agents should contain the following 6 components.

1) Two critical requirements for improved arbovirus surveillance are strengthening of networks (state, national, and international) to detect, control, and reduce emerging diseases and improvement of the capacity to respond to disease outbreaks, especially the improvement of medical and scientific expertise.

2) Surveillance networks should be improved so that they include better electronic communication to and from DHS and MVAC with other states, local health departments, US quarantine stations, health care professionals, and veterinary and wildlife disease organizations. Improved networking is essential to the enhancement of California’s capacity to respond to complex infectious disease threats, including bioterrorism.

3) There must also be a strengthening of disease surveillance and response plans and the development of improved standards and guidelines for such plans. An improvement of public health infrastructures, including laboratories, research facilities, technology, and communication links, are needed to implement these guidelines. Response plans must be developed to ensure prompt implementation of prevention strategies and enhanced communication of public health information about emerging diseases.

4) It is essential that we strengthen both applied and basic research. The investigation and monitoring of vector-borne diseases is essential. Control and treatment must be based on scientific knowledge. The research must focus on understanding the mechanisms of transmission, the natural history of the disease, and the effects of control.
Elements of emerging-disease control plans

If we accept the premise that pathogen and vector introductions constitute potential threats to California, we should next ask what can we do to better prepare ourselves to detect and respond to an introduction? The following section is a series of suggestions, taken from a detailed report by Koplan and Hughes (1998), for fortifying California’s current outstanding surveillance program.

6) Finally, there should be encouragement for other organizations to improve their public health systems and to coordinate activities through national and international organizations.

If these issues can be addressed and solved, the result would be a state-wide and national network for surveillance that should ensure prompt identification of infectious disease outbreaks. Equipment and personnel would be in place to provide appropriate and rapid public health responses to disease threats and bioterrorist incidents. Improved diagnostic methods will allow rapid detection of emerging diseases. Reference reagents would be available for public health and regional reference laboratories. The improved understanding of disease risk factors would lead to improved strategies for disease prevention and treatment. Among the most important outcomes would be that following generations of biologists, epidemiologists, laboratory scientists, and public health officials would be trained and prepared to respond to emerging disease threats.

Acknowledgements

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REFERENCES CITED


Table 1. Enzootic virus activity detected by 3 methods during 1998 and 1999.

<table>
<thead>
<tr>
<th>Year</th>
<th>Method</th>
<th>Coachella Valley</th>
<th>Kern County</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Tested</td>
<td>WEE+</td>
</tr>
<tr>
<td>1998</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1999</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Year</td>
<td>Method</td>
<td>Tested</td>
<td>WEE</td>
</tr>
<tr>
<td>------</td>
<td>---------------</td>
<td>--------</td>
<td>-----</td>
</tr>
<tr>
<td>1998</td>
<td>Mosquito pools</td>
<td>808</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Chicken flocks</td>
<td>10</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>Wild birds</td>
<td>3,329</td>
<td>39</td>
</tr>
<tr>
<td>1999</td>
<td>Mosquito pools</td>
<td>470</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Chicken flocks</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Wild birds</td>
<td>1,021</td>
<td>14</td>
</tr>
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</table>

*Seroconversion occurred between Nov 1998 and Mar 1999*

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Century. Centers for Disease Control and Prevention, US Dept. of Health and Human Services, Atlanta, GA.


*Publication based on research presented in this paper.*

Table 2. Percentage of total birds sampled and total infected with WEE of 6 key species collected in Coachella Valley and Kern County.

<table>
<thead>
<tr>
<th>Species</th>
<th>Coachella Valley</th>
<th>Kern County</th>
<th>Kern County</th>
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<tr>
<td></td>
<td>1998-99 %</td>
<td>1999 %</td>
<td>1997-98</td>
</tr>
<tr>
<td></td>
<td>% WEE total</td>
<td>% WEE total</td>
<td>% WEE total</td>
</tr>
<tr>
<td>House sparrow</td>
<td>7.5 9.4</td>
<td>14.8 0.0</td>
<td>0.8 0.0</td>
</tr>
<tr>
<td>House finch</td>
<td>3.2 9.4</td>
<td>0.1 0.0</td>
<td>23.2 54.6</td>
</tr>
<tr>
<td>Gambel's quail</td>
<td>13.4 42.2</td>
<td>59.7 100.0</td>
<td>nc</td>
</tr>
<tr>
<td>California quail</td>
<td>nc</td>
<td>nc</td>
<td>6.6 18.2</td>
</tr>
<tr>
<td>Common ground dove</td>
<td>7.5 14.1</td>
<td>3.5 0.0</td>
<td>nc</td>
</tr>
<tr>
<td>Mourning dove</td>
<td>4.4 1.6</td>
<td>8.0 0.0</td>
<td>1.4 4.6</td>
</tr>
<tr>
<td>Other species</td>
<td>63.9 23.4</td>
<td>14.0 0.0</td>
<td>68.0 22.7</td>
</tr>
<tr>
<td>Total</td>
<td>10,945 64.7</td>
<td>1,396 13.8</td>
<td>8,021 66.6</td>
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Table 3. Antibody persistence between 1998 and 1999 in recaptured California and Gamble's quail.

<table>
<thead>
<tr>
<th></th>
<th>PRNT* 1998</th>
<th>1999</th>
<th>TIME (wks) **</th>
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<tbody>
<tr>
<td>California quail</td>
<td>160</td>
<td>20</td>
<td>28</td>
</tr>
<tr>
<td>Gamble's quail</td>
<td>160</td>
<td>20</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>320</td>
<td>0</td>
<td>21</td>
</tr>
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</table>

Reciprocal titer of plaque reduction neutralization test
**Time between first positive bleed in 1998 and last recaptured in 1999.

Table 4. Reinfection experiment with house finches. V:V, infected during 1998 and then re-infected with same virus during 1999; V:S, infected during 1998 and then inoculated with saline during 1998; V and S, inoculated with virus or saline during 1998; n = sample sizes for each virus.

<table>
<thead>
<tr>
<th>Time</th>
<th>TREATMENT</th>
<th>WEE</th>
<th>EIA*</th>
<th>PRNT**</th>
<th>Viremia***</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 wks PI</td>
<td>V:V</td>
<td>V:S</td>
<td>V</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>6</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>EIE</td>
<td>3.81</td>
<td>2.65</td>
<td>1.34</td>
<td>1.21</td>
<td></td>
</tr>
<tr>
<td>PRNT**</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td></td>
</tr>
<tr>
<td>Viremia***</td>
<td>0</td>
<td>&gt;4.5(3)</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SLE</td>
<td>EIA*</td>
<td>2.73</td>
<td>2.33</td>
<td>1.06</td>
<td>1.2</td>
</tr>
<tr>
<td>PRNT**</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td></td>
</tr>
<tr>
<td>Viremia***</td>
<td>2.9(3)</td>
<td>0</td>
<td>3.9(3)</td>
<td>0</td>
<td></td>
</tr>
</tbody>
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*mean formula value = mean optical density of positive/negative wells.
**reciprocal of geometric mean titer
***log₁₀ plaque forming units