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ARBOVIRUS TITER VARIATION IN FIELD-COLLECTED MOSQUITOES

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ABSTRACT. Patterns in the distribution of titers in arbovirus-positive mosquito pools were examined. Virus isolation records from the Division of Vector-Borne Infectious Diseases, Centers for Disease Control and Prevention, from 1974 through 1993 were used to estimate virus titers in field-collected pools. Pools were classified as either low titer ($\leq$3.0 $\log_{10}$ plaque-forming units [PFU]/ml) or high titer (>3.0 $\log_{10}$ PFU/ml). The proportion of virus-positive pools that had high titers varied among the different domestic arboviruses, within viruses among field sites and years, and within viruses among mosquito species tested. Alphaviruses produced a greater proportion of pools with high titers than did the flaviviruses. Variation in the proportion of pools with high titers among sites and years suggested variation in mosquito and/or virus strains. Variation in the proportion of pools with high titers among mosquito species indicated species-specific differences in vectorial capacity. The results show that information about the titer of virus in mosquito pools can complement other parameters, such as the minimum infection rate, currently used in mosquito-based arbovirus surveillance programs.

INTRODUCTION

Arbovirus surveillance programs based on virus isolation from mosquito pools have historically used the minimum infection rate (MIR) as an indicator of the intensity of virus transmission activity. The MIR is calculated using the simple formula:

$$\frac{\text{Number of positive pools}}{\text{Total mosquitoes tested}} \times 1{,}000.$$

The calculation produces an MIR expressed as the number of mosquitoes infected per 1,000 tested. High arbovirus MIRs in mosquito populations are frequently associated with outbreaks and with increases in the risk of human disease (Reeves and Hammon 1962, Hayes et al. 1967, Holden et al. 1973, Crans et al. 1986). Although determining the MIR is labor intensive and expensive, it is essential to have data quantifying the proportion of vectors that are infected (in addition to information about vector population density and age structure, host susceptibility, time of year, and weather patterns) when attempting to develop a predictive surveillance program.

Although more precise estimates of virus infection rates can be made (Chiang and Reeves 1962), for practical reasons, the calculation of MIRs generally assumes that only one mosquito is infected in a virus-positive pool. This is a reasonable assumption given the low infection rates that are usually observed in field populations. Though arbovirus titers vary in infected, field-collected mosquitoes (Scrivani and Reeves 1962, Hildreth et al. 1984, Scott et al. 1987), the calculation of MIRs treats all pools containing virus identically, regardless of the titer. The purpose of this study was to compare titers in pools of virus-positive, field-collected mosquitoes, and to examine patterns that may provide information useful to surveillance programs. The virus titers analyzed in this study were estimated using isolation records from studies conducted from 1974 through 1993 by the Division of Vector-Borne Infectious Diseases (DVBID) and its predecessor organization the Division of Vector-Borne Viral Diseases (DVBVD).

MATERIALS AND METHODS

Data were obtained from records prepared by DVBID for each virus isolate derived from field material. Data used in this analysis included the year of the study, state where collected, mosquito species identification, virus identity, tissue culture detection system, and number of plaques in the original sample. From 1973 to 1985, both primary duck embryo (DE) cells and Vero cells were used. After 1985, only Vero cells were used in the tissue culture plaque assay. Virus identity was determined by indirect fluorescent antibody and neutralization tests.

Specimens were prepared for tissue culture plaque assay by grinding mosquitoes in pools of up to 100 individuals in 2 ml of diluent, centrifuging, and inoculating a portion of the supernatant into cells (Mitchell et al. 1987). The volume of supernatant inoculated was 100 µl from 1973 to 1976, and 200 µl from 1977 to 1989. From September 1989 to 1993, 100 µl was inoculated into each of 2 wells in a 6-well plate.

Because these investigations were designed only to detect the presence of virus in field-col-

lected specimens, but not to determine the titer of virus in the samples, the number and appearance of plaques was recorded as shown below:

1–50 Actual number of plaques counted and recorded.
>50 Plaques discernible as separate, but numbered >50.
TNTC Plaques were too numerous to count, probably greater than 100.
CONF Plaques were confluent across the cell layer and impossible to count.
LYSED All cells were lysed.

Therefore, the actual titer of virus in each sample is not known. For our analyses, the pools in which the plaques were countable or were coded as >50 were estimated to have a virus titer ≤3.0 $\log_{10}$ plaque-forming units [PFU]/ml, and were classified as low-titer pools. Pools that were coded as TNTC, CONF, or LYSED were estimated to have a virus titer >3.0 $\log_{10}$ PFU/ml, and were classified as high-titer pools. The number of high and low titer pools was tabulated and analyzed for each arbovirus (St. Louis encephalitis [SLE], eastern equine encephalitis [EEE], western equine encephalitis [WEE], La Crosse encephalitis [LAC], Highlands J [HJ]), for individual arbovirus outbreak investigations, and for arbovirus–vector combinations.

RESULTS

The relative sensitivity of DE and Vero cells to SLE, EEE, and WEE viruses was examined using the McNemar test for significance of change to compare the number of low-titer (≤3.0 $\log_{10}$ PFU/ml) and high-titer (>3.0 $\log_{10}$ PFU/ml) pools using the different cell types (Sokal and Rohlf 1969). In SLE-positive pools, more pools with a high titer were detected with the Vero assay (i.e., for a given infected pool, more plaques were produced on Vero cells than on DE cells) ($P < 0.01$, df = 1, $\chi^2 = 79.0$). Thus, Vero cells were more sensitive in detecting SLE virus. The DE cells appeared to be more sensitive to WEE virus ($P < 0.01$, df = 1, $\chi^2 = 148.4$). No difference in sensitivity to EEE virus was detected ($P > 0.05$, df = 1, $\chi^2 = 0.5$). Therefore, in the following analyses, when a choice was available, Vero cell plaque assay results were used for determining the titer of SLE-positive pools and DE cell results were used for WEE-positive pools. Either DE or Vero results were used for EEE-positive pools, depending on which system produced a higher titer.

A summary of all of the isolates from virus-positive pools from 1974 to 1993, classified by virus, is shown in Table 1. Low-titer pools were common. Virus titer was estimated to be high in only 39% of SLE (Vero)-positive pools, whereas 74% of EEE (DE), and 64% of WEE (DE) virus-positive pools had high titers. The alphaviruses appear to produce more pools with high titers than does SLE. Data are presented for HJ and LAC, but sample sizes are very small.

Table 1. Summary of virus isolates from pools tested from 1974 to 1993, and the proportion of pools with either high or low estimated virus titers.

Virus[1]	Cells	*n*	Proportion of pools: Low titer	Proportion of pools: High titer
SLE	Vero	401	0.61	0.39
	DE[2]	703	0.79	0.21
EEE	DE	159	0.26	0.74
	Vero	184	0.27	0.72
WEE	DE	1,199	0.36	0.64
	Vero	962	0.52	0.48
HJ	DE	39	0.41	0.59
	Vero	39	0.41	0.59
LAC	Vero	8	0.88	0.13

[1] SLE = St. Louis encephalitis, EEE = eastern equine encephalitis, WEE = western equine encephalitis, HJ = Highlands J, LAC = La Crosse encephalitis.
[2] DE = duck embryo.

The distribution of estimated titers of SLE, WEE, and EEE viruses in isolates from field-collected mosquito pools also varied among major investigations conducted by DVBID. In only 45% (5/11) of the major SLE investigations did more than 50% of the isolates have high estimated titers (Table 2). The proportion of high titer isolates in the remaining 6 investigations ranged from 0% to 37%. In 78% (11/14) of the WEE investigations more than 50% of the isolates had high estimated titers (Table 3). In 82% (9/11) of EEE investigations the proportion of isolates in the high-titer range was >50% (Table 4).

Virus titer distribution in positive pools varied among the different mosquito species that were tested. Among the SLE-positive pools, 56% of the *Culex quinquefasciatus* Say pools, 67% of the *Cx. tarsalis* Theobald pools, and 44% of the *Cx. restuans* Theobald pools had high estimated titers (Table 5). High titers were estimated in only 31% of pools from specimens identified as *Cx. pipiens* Linn. In the WEE-positive pools, 66% of the *Cx. tarsalis* pools had high titers, whereas only 29% of *Aedes vexans* (Meigen) pools fell in the high-titer range (Table 5). Among the EEE-positive pools, 87% of *Culiseta melanura* (Coq.) pools, 65% of *Coquillettidia perturbans* (Walker) pools, and 64% of *Ae. al-*

Table 2. Virus isolates from mosquito pools tested in investigations of St. Louis encephalitis virus from 1974 to 1993 and the proportion of pools with either high or low estimated virus titers.

Year	State	Cells	*n*	Proportion of pools: Low titer	Proportion of pools: High titer
1975	TN	Vero	136	0.80	0.21
1975	MS	Vero	14	0.49	0.50
1975	IL	Vero	7	1.00	0.00
1976	TN	Vero	84	0.63	0.37
1979	TN	Vero	19	0.27	0.74
1980	TN	Vero	7	0.43	0.57
1980	TX	Vero	54	0.24	0.76
1983	AZ	Vero	29	0.27	0.72
1984	AZ	Vero	10	1.00	0.00
1985	CA	DE[1]	18	0.78	0.22
1991	AR	Vero	12	0.83	0.17

[1] DE = duck embryo.

Table 3. Virus isolates from mosquito pools tested in investigations of western equine encephalitis virus from 1974 to 1993 and the proportion of pools with either high or low estimated virus titers.

Year	State	Cells	*n*	Proportion of pools: Low titer	Proportion of pools: High titer
1975	CO	DE[1]	43	0.39	0.60
1975	MN	DE	87	0.35	0.66
1975	ND	DE	266	0.53	0.47
1977	CO	DE	257	0.27	0.73
1978	CO	DE	22	0.41	0.59
1979	CO	DE	38	0.37	0.63
1983	AZ	DE	23	0.17	0.83
1983	MN	DE	110	0.25	0.75
1983	ND	DE	231	0.27	0.73
1984	AZ	DE	16	0.13	0.88
1985	CA	DE	18	0.84	0.17
1987	CO	Vero	38	0.34	0.66
1991	CO	Vero	21	0.34	0.67
1993	AZ	Vero	3	0.67	0.33

[1] DE = duck embryo.

bopictus (Skuse) pools had high estimated titers (Table 5).

DISCUSSION

The MIR is an estimate of the proportion of mosquitoes in a population that is carrying detectable amounts of an arbovirus. As such, it is a valuable index that can be used to compare arbovirus transmission activity over time and space. However, quantification and comparison of virus titers in infected pools may also provide surveillance programs with valuable information about arbovirus transmission dynamics.

Using estimated virus titers from historical data, we found that the proportion of virus-positive pools that had high titers varied among the different domestic arboviruses, within viruses among field sites and years, and within viruses among mosquito species tested. Variation in the percentage of pools containing high titers among the different viruses is undoubtedly the result of inherent differences in the infectivity of the viruses. Alphaviruses tend to replicate faster and produce higher titers in mosquitoes than flaviviruses (Chamberlain et al. 1954, 1959), and would produce the observed pattern.

Variation in titers among field sites and years, within viruses, may be due to variation in virus strain or variation in the susceptibility of the local mosquito strain to the virus. For example, epizootic strains of Venezuelan equine encephalitis are more infectious to *Aedes taeniorhynchus* than are enzootic strains (Kramer and Scherer 1976), and SLE viral strains vary in their infectivity for *Cx. quinquefasciatus* (Mitchell et al. 1983). Monitoring virus titers in mosquito pools may detect combinations of virus and vector strains that produce high titers. Such combinations may facilitate virus transmission

Table 4. Virus isolates from mosquito pools tested in investigations of eastern equine encephalitis virus from 1974 to 1993 and the proportion of pools with either high or low estimated virus titers.

Year	State	Cells	*n*	Proportion of pools: Low titer	Proportion of pools: High titer
1980	MI	Vero	17	0.30	0.71
1980	NJ	DE[1]	10	0.30	0.70
1981	MI	DE	36	0.20	0.81
1982	FL	DE	13	0.16	0.85
1983	MI	Vero	33	0.15	0.85
1983	RI	DE	27	0.23	0.78
1984	FL	DE	12	0.33	0.67
1984	RI	DE	10	0.40	0.60
1991	FL	Vero	14	0.36	0.64
1991	OH	Vero	5	0.80	0.20
1993	FL	Vero	5	0.60	0.40

[1] DE = duck embryo.

Table 5. Virus isolates from mosquito pools tested in investigations of St. Louis encephalitis (SLE), western equine encephalitis (WEE), and eastern equine encephalitis (EEE) viruses from 1974 to 1993, separated by mosquito species, and the proportion of pools with either high or low estimated virus titers.

Mosquito species	Virus	Cells	*n*	Proportion of pools	
				Low titer	High titer
Culex nigripalpus	SLE	Vero	4	0.75	0.25
Cx. pipiens	SLE	Vero	147	0.70	0.31
Cx. quinquefasciatus	SLE	Vero	82	0.38	0.56
Cx. restuans	SLE	Vero	9	0.56	0.44
Cx. salinarius	SLE	Vero	14	0.71	0.29
Cx. tarsalis	SLE	Vero	36	0.33	0.67
Aedes vexans	WEE	DE[1]	28	0.72	0.29
Cx. erythrothorax	WEE	DE	2	1.00	0.00
Cx. tarsalis	WEE	DE	1,133	0.34	0.66
Ae. albopictus	EEE	Vero	14	0.36	0.64
Ae. canadensis	EEE	DE	2	1.00	0.00
Ae. vexans	EEE	DE	4	1.00	0.00
Coquillettidia perturbans	EEE	DE	20	0.35	0.65
Culiseta melanura	EEE	DE	113	0.13	0.87

[1] DE = duck embryo.

and amplification, and indicate an increased risk of human disease.

Variation in titer within viruses, among mosquito species, reflects species-specific differences in susceptibility that have been well documented (Hardy 1988). In general, mosquitos must develop a high titer of virus before they are able to transmit, and mosquito species with a high vector competence commonly develop high virus titers (Chamberlain et al. 1959). Therefore, monitoring the titer in virus-positive pools may provide information about the relative importance of different vector species in an area, and about the proportion of infected vectors that are likely to be able to transmit the virus. Monitoring virus titer in pools of competent vector species may also assist in efforts to quantify the progression of the amplification cycle and the effect of environmental factors such as temperature on transmission dynamics.

Although tissue culture plaque assay is a sensitive method for detecting and quantifying arboviruses in mosquito pools, it is a relatively slow and costly process. This has deterred many state and local agencies from conducting mosquito-based arbovirus surveillance programs. Recently, many laboratories have adopted antigen capture enzyme immunoassay (EIA) methods for detecting arboviruses. These EIA techniques are rapid and cost-effective (Hildreth and Beaty 1987), but not as sensitive as tissue culture in detecting virus in low-titer pools (Hildreth et al. 1984, Tsai et al. 1987). Therefore, MIRs calculated using EIA results may not detect low-titer pools, and may underestimate the proportion of virus-infected mosquitoes in the population. However, there are benefits to using EIA that compensate for the lower sensitivity. The amount of viral antigen in a mosquito pool may be rapidly quantified with EIA, allowing analyses such as those described above. In addition, the lower sensitivity of EIA may actually result in a more accurate estimate of the vectorial capacity of a population because the MIR calculated using EIA-positive pools will reflect the proportion of specimens capable of transmitting the virus.

In summary, quantification of virus titer in positive mosquito pools can complement the MIR, the traditionally used index of arbovirus activity. Incorporation of this information into surveillance programs can provide more accurate estimates of the relative risk of disease and refine models used to make decisions regarding implementation of public health interventions.

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