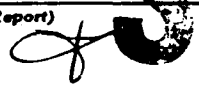


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AN EPIDEMIOLOGICAL STUDY OF HART PARK AND TURLOCK VIRUSES  
IN CALIFORNIA

Thomas G. Ksiazek

ABSTRACT

Turlock (TUR) and Hart Park (HP) viruses were repeatedly isolated from the mosquito Culex tarsalis in California. Both viruses were found to replicate in Cx. tarsalis following parenteral inoculation. Once infected, Cx. tarsalis could transmit HP virus to blood droplets. Low titers of HP stock viruses did not allow efficient evaluation of infection of Cx. tarsalis by ingestion of the virus. Laboratory studies failed to demonstrate transovarial transmission of either virus from parenterally infected females to their progeny. One isolation of HP virus was made from a male Cx. tarsalis collected in Kern County but the frequency of isolation (1/8848) was much less than that from female Cx. tarsalis (112/38,369).

A combination of neutralization and indirect fluorescent antibody tests on sera collected in California from humans and a variety of domestic and feral animals showed that TUR virus infection was most prevalent in feral birds and horses and HP virus infection was prevalent in feral birds and domestic dogs. In spite of previous unpublished reports, no central nervous system disease in horses could be associated with TUR virus infection. Sera from 5 of 1732 human cases of central nervous system diseases had low neutralizing antibody titers to HP virus that increased 4-fold from acute to convalescent

samples. Only 1 of 1,967 human sera was found to have TUR viral antibody. Sentinel chickens were regularly infected by TUR virus in the Central Valley of California while HP virus did not infect sentinel chickens.

Data from a cooperative California arbovirus surveillance program showed Cx. tarsalis minimal infection rates (MIRs) for TUR and HP viruses to be more consistent than those of western equine encephalomyelitis virus from 1978 to 1982. HP and TUR viral MIRs were not consistently dependent on relative abundance of Cx. tarsalis nor were infection rates of sentinel chickens consistently dependent upon TUR and HP MIRs. However, the county-month nature of data for Cx. tarsalis abundance, Cx. tarsalis MIRs, and sentinel chicken infections were probably insensitive measures of the true relationship of these three variables at individual geographical sites.

As expected, linear multiple regression models were more predictive in explaining the variation of TUR and HP MIRs in Cx. tarsalis than any single variable. A large amount of the explained variation was attributable to measures of environmental factors such as photoperiod and temperature.

TUR and HP viruses are apparently dependent upon a mosquito-bird-mosquito cycle for maintenance during the summer season. The mechanism remains unknown by which these viruses overwinter from one mosquito season to the next.

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An Epidemiological Investigation of Hart Park  
and Turlock Viruses in California

By

Thomas G. Ksiazek

B.S. (Kansas State University) 1969

D.V.M. (Kansas State University) 1970

M.S. (University of Wisconsin, Madison) 1976

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Chairman Date

*James I. Hardy* ..... 4/11/84

*David W. Field* ..... 12 April 1984

*Richard W. Cannon* ..... 20 April 1984

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## ABBREVIATIONS

|        |  |
|--------|--|
| AVRU   | Arbovirus Research Unit                  |
| BABS   | bovine albumin borate saline             |
| CDHS   | California Department of Health Services |
| CNS    | central nervous system                   |
| CPE    | cytopathic effect                        |
| DECC   | duck embryonic cell culture              |
| DFA    | direct fluorescent antibody              |
| DMSO   | dimethyl sulfoxide                       |
| EDTA   | ethyldiamine tetraacetic acid            |
| EIA    | enzyme immunosorbent assay               |
| FBS    | fetal bovine serum                       |
| FITC   | fluorescein isothiocyanate               |
| FLA    | Flanders                                 |
| GRL    | Gray Lodge                               |
| HI     | hemagglutination inhibition              |
| HMAF   | hyperimmune mouse ascitic fluid          |
| HP     | Hart Park                                |
| IFA    | indirect fluorescent antibody            |
| i.t.   | intrathoracic inoculation                |
| LLS    | Llano Seco                               |
| LTI    | light trap index                         |
| MAD    | mosquito abatement district              |
| MD     | mosquito diluent                         |
| MEM    | minimum essential medium                 |
| MIR    | minimum infection rate                   |
| Nt     | neutralization                           |
| PBS    | phosphate buffered saline                |
| PBS-FA | PBS for fluorescent antibody             |
| PFU    | plaque forming unit                      |
| PRN    | plaque reduction neutralization          |
| SLE    | St. Louis encephalitis                   |
| TOT    | transovarial transmission                |
| TUR    | Turlock                                  |
| VBCB   | Vector Biology and Control Branch        |
| VRDL   | Virus and Rickettsial Disease Laboratory |
| VSV    | vesicular stomatitis virus               |
| WEE    | western equine encephalomyelitis         |

## SCIENTIFIC AND COMMON NAMES OF ANIMALS

## BIRDS

|               |                             |
|---------------|-----------------------------|
| house finch   | <u>Cardodacus mexicanus</u> |
| house sparrow | <u>Passer domesticus</u>    |
| chicken       | <u>Gallus gallus</u>        |

## MAMMALS

|                            |                                  |
|----------------------------|----------------------------------|
| antelope ground squirrel   | <u>Citellus nelsoni</u>          |
| badger                     | <u>Taxidea taxus</u>             |
| black bear                 | <u>Ursus americanus</u>          |
| blacktail jackrabbit       | <u>Lepus californicus</u>        |
| bobcat                     | <u>Lynx rufus</u>                |
| California ground squirrel | <u>Citellus beecheyii</u>        |
| cattle                     | <u>Bos taurus</u>                |
| cottontail rabbit          | <u>Sylvilagus spp.</u>           |
| coyote                     | <u>Canis latrans</u>             |
| dog                        | <u>Canis familiaris</u>          |
| flying squirrel            | <u>Glaucomys sabrinus</u>        |
| goat                       | <u>Capra hircus</u>              |
| grasshopper mouse          | <u>Onychomys torridus</u>        |
| gray fox                   | <u>Urocyon cinereoargenteus</u>  |
| harvest mouse              | <u>Reithrodontomys megalotis</u> |
| horse                      | <u>Equus caballus</u>            |
| house mouse                | <u>Mus musculus</u>              |
| kangaroo rat               | <u>Dipodomys heermanni</u>       |
| kit fox                    | <u>Vulpes velox</u>              |
| pig                        | <u>Sus scrofa</u>                |
| San Joaquin kangaroo rat   | <u>Dipodomys nitratoides</u>     |
| sheep                      | <u>Ovis aries</u>                |
| squirrel                   | <u>Sciurus griseus</u>           |
| striped skunk              | <u>Mephitis mephitis</u>         |
| white footed mouse         | <u>Peromyscus maniculatus</u>    |

## I. INTRODUCTION

The purpose of these studies was to investigate the natural history of Turlock (TUR) and Hart Park (HP) viruses in California, including investigations of the involvement of man, lower animals, and arthropod vectors.

Both TUR and HP viruses persist each year in populations of Culex tarsalis in California. This mosquito is known to be a vector of western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE) viruses in human and animal populations. Thus, it is highly probable that man and domestic animals are being bitten by mosquitoes infected with TUR and HP viruses. Therefore, it seemed warranted to determine the role of these viruses as possible pathogens of man and animals and to investigate mechanisms which might allow them to be maintained in nature.

The possibility that other arthropod-borne viruses (arboviruses) are involved in the etiology of central nervous system (CNS) disease seems evident when the etiology of encephalitis cases in the United States is reviewed. Between 1960 and 1978, 43,025 cases of encephalitis were reported (CDC, 1981). Of these, 13% were caused by arboviruses (WEE, SLE, California encephalitis, or eastern equine encephalomyelitis), 1% by enteroviruses, 33% were associated with childhood viral infections such as measles and mumps, and 4% were due to other diagnosed causes. However, 56% of these cases were not diagnosed as belonging to any of the above categories. A high proportion of these undiagnosed cases occurred in the summer which would be compatible with an arboviral etiology.

## OBJECTIVES

The present study had 5 primary objectives:

1. to generate and collate data on the evidence of past infection with TUR and HP viruses in vertebrate populations as measured by an indirect fluorescent antibody (IFA) test in order to determine which species may be important in the natural history of these viruses,
2. to collate data on the frequency of isolation of TUR and HP viruses from vectors, the prevalence of antibodies in sentinel chicken flocks, and the relative abundance of Cx. tarsalis so that the effect of relative vector abundance upon infection rates and transmission rates of the viruses might be determined,
3. to determine if transovarial transmission (TOT) allows TUR and HP viruses to persist in California at a stable level,
4. to evaluate the sensitivity and specificity of the IFA test as a tool in both serological survey and diagnostic studies of TUR and HP viruses, and
5. to develop a general model of the natural history of HP and TUR viruses.

## HISTORICAL BACKGROUND



Arboviruses have been of concern in California since the 1930's and 1940's when WEE and SLE viruses were first found to be associated with significant human disease in California and their mode of transmission by mosquitoes was demonstrated (Reeves, 1976). Subsequent studies further documented the health hazard that SLE and WEE viruses pose and demonstrated the value of vector reduction in disease prevention (Olson et al, 1979). Much of the marked suppression in the level of transmission of these viruses in recent years can be attributed to a large extent to vector control (Reeves and Milby, 1979).

The role of HP and TUR viruses as causes of human or animal disease is not known. TUR virus has been associated with encephalitis in horses on the basis of serologic tests of paired sera from horses (unpublished [1]). Tests on paired sera from human cases of CNS disease have given no indication of an association with TUR infection. The association of HP with disease in man and animals has not been explored except for tests of small numbers of sera.

An intensive surveillance system was initiated in California in 1969 to monitor the activity of these 2 viruses and to direct control measures in a timely fashion should an increase in the level of activity of either of these viruses be detected (Reeves and Milby, 1980). The program includes measurement of the population levels of known vector species, tests of mosquito pools for viral infection,

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1. Unpublished shall hereafter refer to the unpublished data of the University of California School of Public Health Arbovirus Research Unit(AVRU) and its various collaborators.

tests of sera from sentinel chicken flocks for antibodies to indicate viral transmission, and diagnostic serology on sera from humans and horses with CNS disorders submitted to the California Department of Health Services (CDHS) Viral and Rickettsial Disease Laboratory (VRDL).

Several observations can be made from recent surveillance efforts. Transmission of SLE and WEE virus has been at a low level since 1969 (Reeves and Milby, 1979; Emmons et al, 1979; Emmons et al, 1980; Emmons et al, 1981; Emmons et al, 1982; Emmons et al, 1983 [2]). It cannot be stated categorically whether this was due to vector control efforts alone or if other changes in the basic ecology of these viruses contributed to the decline. HP and TUR viruses have been isolated with regularity over the last 5 years while at the same time WEE and SLE, the other viruses associated with Cx. tarsalis, have remained at a relatively low level in most areas (Emmons series).

TUR and HP viruses are both being maintained in many parts of California by Cx. tarsalis populations in which WEE and SLE viruses are not detectable and this has allowed an interesting comparative study to be made on the persistence of these arboviruses. These observations indicate that for viral persistence there must be differences between TUR and HP and WEE and SLE viruses in: 1) the mechanisms for transmission of the viruses from generation to generation of mosquito, 2) the efficiency of vertebrates as reservoirs of infection, or 3) the dependence on relative abundance of vector or

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2. Hereafter referred to as Emmons series

host populations for continuous transmission.

Turlock Virus.

TUR virus was first isolated from a pool of Cx. tarsalis collected in 1947 at a site near Turlock, California and was recognized to be an antigenically distinct virus a number of years later (Lennette et al, 1957; Lennette et al, 1957a).

Viruses identified as TUR are widely distributed geographically and have been isolated repeatedly in California during investigations of SLE and WEE viruses (Emmons series; unpublished). The reported range for the virus extends from Alberta, Canada in the north (Hall et al, 1968) to the Caribbean and South America in the south (Shope et al, 1966, Spence et al, 1968).

TUR virus is known to infect a wide variety of vertebrate species and produces viremia in birds (house finches, house sparrows, Brewer's and tri-color blackbirds, and doves) (Hardy, cited in Berge, 1975). This has led to the belief that birds are the major vertebrate hosts. House finches not only produced sufficient viremia to infect Cx. tarsalis but also became persistently infected with TUR virus (unpublished). Virus has been recovered from the organs of such persistently infected house finches but Cx. tarsalis that fed on birds with chronic infections failed to become infected (unpublished). Therefore, the mechanism for viral persistence in the natural cycle remains unknown. A recent study detailed the response of house

sparrows, bob-white quail, chukar partridge, ring-necked pheasants, chickens, and Japanese quail after laboratory inoculation with TUR virus (Scott et al, 1983). One day old chickens were the only species that responded with detectable viremia. Japanese quail did not develop antibodies and only 1 of 13 bob white quail responded with detectable neutralizing antibody. The majority of birds of the other 4 species responded with neutralizing antibody. In another study, the same group demonstrated that Cx. tarsalis became infected with TUR virus after feeding on viremic house sparrows (Scott et al, 1983a). The movement of infected migratory birds, which are capable of becoming viremic intermittently, would help to explain the wide geographical distribution and the persistence of the virus. Thus, the 2 alternatives for viral maintenance involving birds are reintroduction of virus by viremic migratory species or recrudescence to a viremic state of chronically infected local resident species (Reeves, 1974).

TUR virus hemagglutinates avian erythrocytes and thus the hemagglutination inhibition (HI) test has been used routinely to detect antibodies in animals. The neutralization (Nt) test has been used to a more limited extent. The HI test is not as sensitive as the Nt test in detecting past infections with TUR virus in horses (unpublished). It is not clear if this is due to a short duration of HI antibody, a low quantitative response, or the relative lack of sensitivity of the HI test for Bunyavirus antibodies. A recent study (Beaty et al, 1982) found that the HI and the IFA were nearly as sensitive as the Nt or the enzyme linked immunoassay (EIA) tests for

the detection of LaCrosse virus (genus Bunyavirus) antibodies in human sera from recent clinical cases. However, in sera of clinical cases in which infection had occurred in the more distant past, the HI and IFA tests did not detect antibody with the same efficiency as the Nt or EIA.

Intracranial inoculation of monkeys with TUR virus produced a febrile response but no CNS disease (Behbehain et al, 1967). As was stated above, rising titers of antibody to TUR virus have been found in a few cases of encephalitis in horses (unpublished). Paired sera from humans with CNS disease have been tested by HI without indication that their disease was associated with TUR infection (unpublished). Human infection is not common if the very low rate of HI antibody in the diagnostic sera can be taken as a crude estimate of prevalence (unpublished). HI tests of sera from a number of wild animal species have indicated the general prevalence of past infection. Birds had a higher level of past infection (3% of 4,868 birds of 22 species) than ground dwelling wild mammals such as lagomorphs, heteromyids, and sciurids (0.5% of 2,331) (Hardy et al, 1977; Hardy, cited in Berge, 1975). One isolate of TUR virus was obtained from a jackrabbit in Hale County, Texas (Hayes et al, 1967). House finches had the highest prevalence (33%) of HI antibodies of the wild birds for which reasonable numbers were tested (unpublished). Surveys of domestic mammals resident in California in 1968 indicated that infections do occur. Horses had the highest prevalence of HI antibody (26.3% of 504) followed by cattle (2.6% of 401), and sheep (2.6% of 459) (unpublished). Small numbers of sera from other domestic mammals were

tested and yielded the following percent of positives: goats (0% of 9), pigs (17.4% of 69), and dogs (25.5% of 47).

Prior to 1979, sentinel chickens maintained in the Sacramento and San Joaquin Valleys were tested for TUR Nt antibody. Acquisition of antibody occurred on a regular basis among most sentinel flocks in both locations (unpublished). Seasonal antibody conversion rates in some flocks were over 50% while a few flocks had no conversions. Between 1974 and 1976 the average seasonal conversion in all flocks in the Sacramento valley (691 chickens) was 23.6% while in the San Joaquin Valley (644 chickens) it was 22.2%.

TUR virus is now classified as a member of the family Bunyaviridae and the genus Bunyavirus (Klimas et al, 1981). A closely related virus, Umbre, infects man in Malaysia (Wallace et al, 1977). Umbre virus is also thought to be maintained in a Culex spp. mosquito-bird cycle of transmission. Other Bunyaviruses, primarily members of the California serogroup are at least partially maintained by TOT (Watts et al, 1974; Tesh, 1980; Turell et al, 1982). It is not established if this is a general quality of Bunyaviruses or whether the fact that Aedes mosquitoes serve as primary hosts for most of these viruses determines the ability of the virus to be transmitted in this fashion.

Hart Park virus.

HP virus was first isolated from a pool of Cx. tarsalis collected at Hart Park, Kern County, California in 1955 (Berge, 1975). The virus is antigenically related to Flanders (FLA) an arthropod-borne virus isolated in the Eastern United States (Whitney, 1964). The mosquitoes associated with HP and FLA viruses feed primarily on birds. Additionally, both of these viruses have been isolated from the tissues and blood of various avian species (Whitney, 1964; Kokernot et al, 1969; Johnson cited in Berge, 1975). This may indicate there is a mosquito-bird-mosquito cycle, although unlike TUR virus, there is no evidence to indicate that birds produce sufficient viremia to infect feeding mosquitoes.

HP and FLA viruses have a wide geographical distribution within the United States that extends from the Northeastern and Southeastern Seaboards to the West Coast and from Illinois to Southern Texas. FLA viral isolations have been frequent in surveys associated with investigations of SLE outbreaks. FLA virus has been isolated from Culiseta spp. (Whitney, 1964), Psorophora spp. (Hayes et al, 1976), and Culex spp. mosquitoes (Whitney, 1964; Kokernot et al, 1969; Kokernot et al, 1974; Hayes et al, 1976) while HP virus has been isolated mainly from Cx. tarsalis. One recent isolation of HP virus was reported from Culex erythrothorax in California (Emons et al, 1983) and one reported isolation of HP (FLA?) virus was reported earlier from Aedes nigromaculis collected in Texas (Hayes et al, 1967). HP virus has been isolated from 3 pools of Aedes melaninon collected in Kern County but the status of these isolates is questionable (Emons, personal communication, 1982).

There are no published reports of attempts to transmit HP virus transovarially. However, FLA virus was not isolated from 9,138 male Culiseta melanura and 4,893 male Culex restuans while isolates were obtained from females of the same species collected in the period 1976 to 1979 in Connecticut (Main, 1981). Transovarial transmission has been demonstrated for other rhabdoviruses, most notably vesicular stomatitis virus (VSV) in sandflies (Tesh et al, 1972, Tesh and Chaniotis, 1975) and Sigma virus in Drosophila (Brun and Plus, 1980).

Laboratory studies with HP and FLA virus have been somewhat limited, probably because no simple immunological test such as the HI test is available. There are discernible antigenic differences between HP and FLA strains in Nt tests (Boyd, 1972). Boyd's analyses indicated that FLA virus had a wide geographical distribution while HP virus was largely limited to the western United States. An abbreviated series of comparisons of California HP viral isolates indicate that the strains tested were truly HP and not FLA-like (unpublished).

Both HP and FLA viruses are classified as members of the family Rhabdoviridae on the basis of electron microscopic studies (Murphy et al, 1966; Jenson et al, 1967; Jenson et al, 1971). Although these viruses appear similar to other rhabdoviruses, there is no apparent antigenic relationship to either the rabies group, genus Lyssavirus, (Shope, 1975) or the VSV group, genus Vesiculovirus, (Berge, 1975). Because of this lack of antigenic relationship, both HP and FLA are currently included in an unclassified group of rhabdoviruses (Brown et al, 1979).

There was no evidence that viremia developed in experimentally



inoculated chickens, rabbits, or guinea pigs (Berge, 1975). Chickens inoculated by the intracranial route with FLA virus produced neutralizing antibody (Whitney, 1964). HP virus did not replicate in wild rodents or lagomorphs following inoculation with an isolate of the virus (63V-162) from Texas (unpublished).

Little is known about the prevalence of HP or FLA antibodies in the human or animal populations. In the Ohio-Mississippi Basin, a few persons were reported to have equivocal results for Nt antibody assays in mice (Kokernot et al, 1969). These sera were collected from persons residing in areas where FLA virus frequently was isolated from both birds and Culex spp. mosquitoes. The lack of detectable neutralizing antibody in the sera of birds of the same species as those from which isolations of FLA virus had been made was somewhat puzzling. In California, very limited serological studies employing the Nt test and a local strain of HP virus isolated in the Sacramento Valley (BFNS662) have shown that sciurids, horses, and pigs may have been infected with HP virus (unpublished). A few sentinel chickens appeared to have Nt antibody to HP virus.

A limited attempt has been made to demonstrate the transmission of HP virus in Cx. tarsalis following intrathoracic (i.t.) inoculation (unpublished). The virus replicated in the mosquitoes by 14 days (but not by 7 days) postinoculation but the virus was not transmitted when the mosquitoes were fed on droplets after 14 or 21 days extrinsic incubation.

## II. LABORATORY INVESTIGATIONS OF VIRUSES AND VECTORS.

### INTRODUCTION

This section deals largely with my experimental studies upon HP virus. Turlock TUR virus has been studied to a much greater extent by others and the information available from those studies as well as the limited investigations performed in these studies will be discussed in parallel with those on HP.

### MATERIALS AND METHODS.

#### Viruses.

The history of the HP and TUR viruses used in these studies is presented in Table II-1. The HP stock virus used most extensively, BFN5662, only had a titer of approximately  $4.0 \log_{10}$  PFU/0.1 ml after 8 total passages in Vero cells. A virus which had been passaged 27 times in Vero cells had a titer of approximately  $5.5 \log_{10}$  PFU/0.1 ml. The prototype strain, HP (AR70), did not plaque well even on Vero cells, the only cell on which it produced plaques. This strain could not be passaged in Vero cells even in 2 blind passage attempts. HP (AR70) produced antigen in infected C6/36 cells, but the virus spread

very slowly from cell to cell. The Cx. tarsalis cell line of Chao and Ball was not evaluated for production of HP (AR70) virus and the general lack of success in working with the virus and its rather extensive passage history in mice lead to abandonment of this strain in favor of HP (BFN5662) as the primary virus for use in experimental studies.

Plaque reduction neutralization (PRN) tests were used to confirm the identity of the TUR (847-32), TUR (FMS4783), HP (AR70), and HP(BFN5662) stock viruses. The antisera used in these assays were obtained from the National Institutes of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland and were to the HP (AR70) and TUR (847-32) strains of the viruses. The identities of TUR (Kern 82-63) and HP (Kern 83-5053) viruses were confirmed by the use of direct fluorescent antibody (DFA) tests with conjugates to the HP (AR70) and TUR (847-32) strains.

#### Virus assay.

Preformed cell monolayers were grown in plastic tissue culture plates, rinsed free of growth medium with phosphate buffered saline (PBS) (Dulbecco and Vogt, 1954), and inoculated with 0.1 ml of serially diluted virus stock. After viral adsorption for 1 hour at 35-37 C, the monolayers were rinsed again with PBS and an overlay was placed on the cells to confine virus replication to focal areas. Numerous methods of assay were evaluated but the final method used a

viscous semisolid overlay for both viruses. For HP, Vero cells were used and the overlay was 1.5% methyl cellulose in Eagle's minimum essential medium (MEM) with modified Earle's salts to which 2% fetal bovine serum (FBS), 2 mM L-glutamine, and 20 mM NaHCO<sub>3</sub> were added. A second overlay, in which 1.5% Noble agar was substituted for the methyl cellulose and to which a final concentration of 1:25,000 neutral red was added, was placed on top of the first overlay 4 days after infection.

For TUR virus, duck embryonic cell cultures (DECC) were used with an overlay of 1% special agar in the same MEM base. However, problems with the long term maintenance of DECC under the agar overlay forced the substitution of 1.5% carboxy methyl cellulose (DeMadrid and Porterfield, 1969) with subsequent fixation with formalin and staining of the cell monolayer with crystal violet for plaque visualization.

Mosquito susceptibility to infection with HP virus was determined by i.t. inoculation (Rosen and Gubler, 1974) of Cx. tarsalis (Knight's Landing colony) with 10-fold dilutions of HP (BFN5662) virus. Following 1 week extrinsic incubation at 27 C, the mosquitoes were triturated individually and tested by plaquing on Vero cells. The amount of virus necessary to infect half of the mosquitoes (MID) was interpolated (Reed and Muench, 1938).

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Replication of HP and TUR viruses in selected cell cultures and mosquitoes.

A number of cell lines was evaluated for their ability to support HP viral replication and, in some cases, TUR virus was tested in parallel. The basic approach was to grow a cell line to confluency in 1 ml of growth medium in stationary roller tubes, pour off the growth medium, and then infect the monolayers with 0.1 ml of the stock virus containing approximately  $2.0 \log_{10}$  of virus. After 1 hour adsorption, 1 ml of maintenance medium was added to each tube. Medium blanks were inoculated at the same time to evaluate viral decay in the medium of choice at the temperature of the experiment. Triplicate replicates were withdrawn at predetermined intervals, 0.5 ml of 0.75 % bovalbumin in borate saline (pH 9.0) (BABS) was added to the tube immediately before it was frozen at -70 C. The tubes were then thawed and frozen for 1 additional cycle and the contents assayed by plaquing on Vero cells or primary DECC for HP and TUR viruses, respectively.

Growth curves were performed in mosquitoes from the Knights Landing colony of Cx. tarsalis. In some instances, the growth curve was part of another experiment on TOT. Mosquitoes were infected by i.t. inoculation (Rosen and Gubler, 1974) and then placed at the desired temperature and humidity conditions. From 5 to 10 mosquitoes were removed and frozen at selected days post-inoculation. Individual mosquitoes were triturated in a mosquito diluent (MD) that consisted of PBS with 20% heat inactivated FBS, 100 ug/ml gentamicin, and 100 units/ml of mycostatin and frozen at -70 C until assayed by plaquing as described above.

Mosquito rearing and husbandry.

Adult mosquitoes used in inoculation, feeding, or TOT experiments were used when 3-5 days post-emergence. The Knights Landing colony of Cx. tarsalis was reared under standard conditions (Hardy et al, 1980). Briefly, the colony was maintained at 27 C and 80% relative humidity. Oviposition was stimulated by offering chicks as a blood source and collecting eggs 3-4 days later. Egg rafts were removed, sterilized in a sodium hypochlorite solution and placed in 26.7 by 20.3 by 6.4 cm polyethylene containers (flats) containing deionized water with a small amount of a 5% suspension of a staple fish food (Tetramin). Three days later larval populations were adjusted to approximately 300-400 per flat and then offered a slurry of the fish food until the appearance of 3rd instar larvae. The larvae were then switched to a diet of 2 parts rabbit chow, 2 parts tetramin, and 1 part brewer's yeast and the water was aerated continuously. Pupae were picked and transferred to 3.8 l cardboard containers for emergence. Adults were offered raisins as a carbohydrate source and wetted cotton pads for water. Lower temperature experiments were performed in an environmental chamber maintained at 18 C and 80% relative humidity. For all experiments, photoperiod was maintained at 16 hrs light and 8 hrs darkness with a 0.5 hour artificial dawn and dusk.

Tests for CO<sub>2</sub> sensitivity in mosquitoes infected  
with HP or TUR virus

A number of mosquitoes were removed from growth curve experiments and exposed to CO<sub>2</sub> (Rosen and Shroyer, 1981). Mosquitoes were tested for their infection status and the effect of exposure to CO<sub>2</sub> on their locomotion. Exposure to CO<sub>2</sub> was done 1 week post-infection, a time when virus was at or near peak titers. Mosquitoes from the Knights Landing colony were evaluated for induction of CO<sub>2</sub> sensitivity with the New Jersey, Ogden strain of vesicular stomatitis virus (VSV), 1 week after their i.t. inoculation. This was done to confirm that sensitivity to CO<sub>2</sub> would develop in this mosquito population with a known sensitivity inducing virus (Rosen, 1980). Mosquitoes were tested for the presence or absence of VSV virus by plaquing on Vero cells.

Transmission of HP virus by Cx. tarsalis.

Transmission of HP virus by a mosquito had not been demonstrated. The Knights Landing strain of Cx. tarsalis was evaluated for its ability to transmit HP (BFN5662) virus to a hanging droplet of equal volumes of 50% washed rabbit erythrocytes, 10% sucrose, and FBS (Gubler and Rosen, 1976). Mosquitoes were infected by the i.t. route and transmission was attempted after 14 or 21 days extrinsic incubation at 27 C. Droplets were tested for virus by plaque assay on Vero cells within minutes after each mosquito had fed. Inability to passage the virus from plaques that developed dictated

the use of a DFA technique for viral antigen detection in a cell line derived from Aedes albopictus, C6/36 cells. A direct fluorescein isothiocyanate (FITC) conjugated antibody to HP (AR70) virus was used.

Attempt to infect Cx. tarsalis by the oral route.

An attempt was made to infect the Knights Landing strain of Cx. tarsalis by allowing the mosquitoes to feed on a pledget soaked with a mixture of 1:1:1 10% sucrose, FBS, and 50% rabbit red cells that contained approximately 1.6 log<sub>10</sub> HP virus per 0.003 ml. An aliquot of the virus-blood mixture was diluted 1:2 in BABS, frozen at -70 C, and subsequently assayed to determine the titer of virus ingested by the feeding mosquitoes. Mosquitoes that engorged were removed from the original container and maintained at 27 C for 10 days. Surviving mosquitoes were then triturated individually in MD and assayed on Vero cells as described above.

Transovarial transmission TUR and HP viruses.

The basic protocol for TOT experiments was to infect females by i.t. inoculation. The viruses used in early experiments were HP (BFN 5662) and TUR (FMS 4783). Later studies utilized a field strain of



each virus which had received only 1 or 2 i.t. passages in the Knights Landing colony of Cx. tarsalis; these viruses were HP (Kern 83-5350) and TUR (Kern 82-63). Inoculated mosquitoes were incubated for 7 days prior to offering them a chick as a blood source to stimulate egg production. Fed mosquitoes were held an additional 5-7 days when an oviposition site was provided. Eggs were hatched and progeny maintained on the diets described earlier. In some experiments both maternal females and larval progeny were maintained at 18 C, a temperature shown to increase the efficiency of SLE virus TOT (Hardy et al, 1980). In other experiments, 27 C was used for maintenance.

Larval or adult progeny to be tested for virus were counted to form pools of no more than 25 individuals. Adults were also sorted according to sex. Larvae were washed with deionized water by placing them in a tube with a mesh bottom and rinsing thoroughly. Larvae were then placed in 4 ml plastic tubes, drained, and frozen at -70 C. Both adult and larval pools were triturated in 2 ml of BABS in a Ten Broeck grinder, and then centrifuged at 10,000 X G for 20 minutes at 5 C. The supernatant fluid was stored in a plastic tube at -70 C until assayed for virus. A pool size of no more than 25 larvae and the use of a high pH buffer, BABS, as the diluent were dictated by studies which indicated that normal larval tissues could render considerable amounts of virus undetectable (Ksiazek et al, 1983).

Inoculation of chicks with HP virus.

In previous studies, no laboratory hosts could be infected with HP (63v-162) (unpublished). However, Whitney, (1964) had reported that neutralizing antibody was detected in 8 hour old chicks that were inoculated with a closely related rhabdovirus, FLA. In the present study, 5 day old chicks were inoculated with from 2 to 3 log<sub>10</sub> of HP virus by the intravenous (0.1 ml), subcutaneous (0.1 ml), or intracranial (0.025 ml) route. Virus was diluted in PBS with 0.5 % gelatin added. The chicks were bled 3 weeks post-inoculation and tested for Nt and IFA antibodies.

## RESULTS

### Plaque assay of HP and TUR viruses in selected cell cultures

Several methods were evaluated to determine the optimal system to assay HP and TUR viruses (Table II-2). TUR virus plaqued in a wide variety of cells while HP only plaqued in Vero and BHK (0853) cells. Ultimately, Vero cells were selected as the best assay system for HP virus and DECC for TUR virus.

Mosquito inoculation had been found previously to be approximately 100 times more sensitive than plaque assay in cell culture to detect infection with the same stock of TUR virus on a volume per volume basis (unpublished). Therefore, a comparison of the

sensitivity of inoculation of Cx. tarsalis (Knights Landing colony), plaquing on Vero cells, and infection of the C6/36 line of Ae. albopictus cells was done with HP virus (Table II-3). Again, mosquito inoculation was more sensitive for viral detection but the volume difference inherent to inoculation of a mosquito versus a cell culture (0.00017 ml vs. 0.1 ml) more than compensated for this deficiency. Thus it appeared that, when volume is considered, the Vero cell system was as sensitive as any method evaluated.

Replication of HP and TUR viruses in selected cell cultures and mosquitoes.

The dynamics of virus replication of the BFN5662 and AR70 strains were determined following i.t. inoculation of Cx. tarsalis and incubation at 27 C (Figures 1 and 2). HP (AR70) virus did not seem to replicate to the same high titers in Cx. tarsalis (Figure 1) as did HP (BFN5662) virus (Figure 2). Maximum mean titers for both AR70 and BFN5662 viruses were reached at 10 days post-inoculation. However, there was a more rapid increase in viral titer for BFN5662 virus with a sharp break in the increase of titers at day 4. AR70 increased in viral titer for a more prolonged period and its maximum titer was reached on day 10. AR70 virus subsequently showed a gradual decrease in viral titer.

The growth of HP (BFN5662) and TUR (FMS4783) viruses also was evaluated at an extrinsic incubation temperature of 18 C. As expected,

the growth rate of HP (BFN5662) virus was slower at 18 C (Figure 3) than at 27 C (Figure 2). The peak titer for HP (BFN5662) virus at 18 C was reached at approximately 10 days and then gradually declined through day 48. TUR (FMS4783) virus grew much better than HP (BFN5662) virus since nearly maximum titers were attained 2 days after inoculation (Figure 4). The mean titers fluctuated within a range of 3.5-4.0 log /mosquito from day 2 through 24.

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HP viral growth also was assayed in 1 avian (DECC, Figure 5), 2 mammalian (BHK, Figure 6; Vero, Figure 7) and 2 mosquito (Figures 8 and 9) cell systems. Interestingly, the most consistent system for HP viral replication seemed to be a Cx. tarsalis cell line. The virus multiplied to higher titers and with a much higher degree of consistency in these cells than in any of the other systems evaluated, including Aedes albopictus cells. Assays of medium controls indicated a relatively rapid viral decay at 37 or 28 C. No cytopathic effect (CPE) was observed in the mosquito cells during the growth curve experiments. Vero and BHK cells both exhibited CPE but the extent of cellular degeneration was limited and did not encompass all of the cells in the cellular sheet.

In contrast, TUR (847-32) virus grew quite well in both DECC (Figure 10) and C6/36 (Ae. albopictus) cells (Figure 11). Peak titers occurred on day 2 in DECC cells as compared with day 6 in C6/36 cells. This could reflect the different incubation temperatures for the 2 cell systems. TUR virus grew slowly in Vero cells and viral titer did not seem to have reached a peak at the end of the 7 day growth experiment (Figure 12). Growth of TUR virus in BHK and Cx.

tarsalis cells was not evaluated.

Transmission of HP virus by Cx. tarsalis.

Attempts were made to determine if Cx. tarsalis infected by the i.t. route could transmit virus to hanging droplets. In experiment I, plaques were demonstrated in 11/47 (23%) of the droplets inoculated onto the Vero cells (Table II-4) but the number of plaques visualized was very small, many showing only 1 or 2 plaques with the maximum number being 13 plaques. Attempts to recover the virus for serological identification were unsuccessful. Therefore, another experiment, experiment II, was performed in which the presence of virus in droplets on which the mosquitoes had fed were inoculated into C6/36 cells which were incubated for 7 days at 28 C before being tested for HP antigen by DFA test (Table II-4). Virus antigen was visualized in 8 of 19 (42%) samples of mosquitoes that had taken a full or partial blood meal 14 days post-inoculation. All mosquitoes which had fed in both experiments were infected with virus as determined by plaque assay on Vero cells.

Attempts to infect Cx. tarsalis with HP virus by the oral route.

Two experiments were performed in which a total of 46 female Cx.

tarsalis were judged to have fed to at least 1/2 of total repletion on pledgets soaked with HP (BFN5662) virus. The mean virus titer ingested in these experiments was approximately 1.6 log<sub>10</sub> or 40 pfu per mosquito. Fed females were tested after 9 days incubation at 27 C for virus content by plaque assay on Vero cells and no virus was detected. The findings are not surprising considering the relatively small amount of virus the females had ingested.

Tests of transovarial transmission TUR and HP viruses by experimentally infected Cx. tarsalis.

The first experiments evaluated hypotheses of TOT of TUR and HP viruses from i.t. inoculated female Cx. tarsalis (Knights Landing colony) to immature stages of progeny. No viral isolations were made from larval progeny of females infected with either TUR (Table II-5) or HP (Table II-6) virus. In both experiments a sufficient number of 4th instar larvae were assayed to reject the hypothesis at the 0.05 level of probability. All of the maternal females from which these progeny were derived were found to be infected.

One attempt to demonstrate TOT of HP(BFN5662) virus to larval and adult progeny incubated at 27 C was negative (Table II-7). All (20/20) of the female mosquitoes tested after inoculation were positive for HP viral plaques.

One attempt to demonstrate TOT of a field strain of TUR (Kern 82-63) virus in Aedes epactius was unsuccessful. This species had

been shown to transmit SLE virus transovarially (Hardy et al, 1980) (Table II-8). Although the numbers of progeny assayed for viral transmission in the Ae. epactius experiment were not as large as in the previous experiments, they were sufficient to reject the null hypotheses that the proportion of infected progeny was considerably smaller than that found for other Bunyaviruses such as LaCrosse and California encephalitis viruses (Turell et al, 1982a; Miller et al, 1982).

TOT experiments were completed with a field strain of HP virus (Kern 83-5350) in a field population of Cx. tarsalis collected from the same location at which the viral isolate had been made (Table II-9). This virus had been passaged 1 time in the Knights Landing colony of Cx. tarsalis. Only a few progeny mosquitoes were obtained because of severe mortality of parental females at about the 10th day post inoculation. There was no evidence of TOT to 1,729 adult progeny tested. All parental females from which progeny were reared were infected. An attempt was made to repeat this experiment using the same HP virus after it had received 1 further i.t. passage in females from the Knights Landing colony. Similar mortality problems were encountered subsequently with parental females inoculated with this virus.

Further experiments were planned in which recently isolated field strains of HP and TUR viruses would be inoculated into field collected mosquitoes so as to rule out laboratory adaptation of the virus or the mosquito as factors that might block TOT. However, high mortality problems developed that were associated with an unidentified

filterable agent in viral stocks and the experiments were abandoned.

CO sensitivity tests of mosquitoes infected with virus  
2

During several experiments TUR or HP infected Cx. tarsalis were subjected to chilling and CO exposure and there was no evidence of CO sensitivity. An occasional mosquito exhibited limited ataxia but in no instance did CO anesthetized mosquitoes have a higher proportion affected than uninfected control mosquitoes. Aedes melaninon females infected with HP (BFN5662) virus also were not sensitive to the effects of CO. All CO sensitivity tests were done at 7 days post-inoculation when HP virus was nearing peak titers and by which time TUR virus had reached peak titers. The Knights Landing colony was CO sensitive following inoculation with VSV. All 21 mosquitoes judged to be sensitive to CO yielded typical VSV plaques. Four mosquitoes judged to be CO non-sensitive were not infected. This experiment demonstrated that the methods and mosquitoes employed in these studies would detect CO sensitivity.

Inoculation of chicks with HP virus.

Two attempts were made to infect baby chicks with HP virus. In the first, 2 groups of 5 (5 day old) chicks each were inoculated by



the intravenous and subcutaneous routes with  $2.5 \log_{10}$  of HP (BFN5662) virus. None had measurable Nt or IFA antibody 3 weeks post-inoculation. Five 1 day old chicks were inoculated intracranially with approximately  $2.0 \log_{10}$  of either HP (BFN5662) or HP (AR70) virus and none developed detectable antibody.

## DISCUSSION

### Replication of viruses.

The replication of TUR virus is like that of most arboviruses in a wide range of vertebrate and invertebrate cell lines. TUR virus also plaqued on the majority of vertebrate cell lines tested. On the other hand, the titers of HP virus attained in a number of cell lines were relatively low and plaque production was limited to the Vero and BHK (0853) cell lines. The best titers of HP virus were obtained in a cell line derived from an apparent natural vector, Cx. tarsalis.

Both HP and TUR viruses replicated in Cx. tarsalis following i.t. inoculation. The pattern of replication was typical for viruses artificially inoculated into mosquito hemocoelae. The AR70 strain of HP virus replicated to lower titers than the BFN5662 strain; this finding may be explained by the high passage of AR70 in mice (19 passages) which could have selected for variants in the viral population that grow poorly in mosquitoes. Whitman maintained HP

virus by serial passage through Ae. aegypti (cited in Berge, 1975). In these experiments, the virus was passaged by removal of the salivary glands of the mosquito, indicating that the original virus had replicated and there was a potential for viral transmission by bite. However, the possibility that dissected salivary glands were contaminated with virus from hemolymph could not be discounted.

HP virus was transmitted by female Cx. tarsalis which had been infected by i.t. inoculation 14 to 21 days earlier. Droplet feeding was used to evaluate transmission and this method had been found to be relatively inefficient as compared with detection by feeding on susceptible hosts to detect transmission of SLE and WEE viruses (unpublished). Transmission of HP virus by feeding on a suitable vertebrate host would have been a preferable method but no such host has been identified. The droplet feeding technique was the only available way to evaluate the potential for virus transmission at this time. The effect of length of extrinsic incubation or effect of temperature upon transmission was not investigated beyond the 2 periods of 14 and 21 days at 27 C.

The transmission of TUR virus by Cx. tarsalis has been investigated more extensively (unpublished) and this mosquito becomes infected by feeding on house finches and can complete the infection cycle by transmitting TUR virus to susceptible house finches.

Attempts to infect Cx. tarsalis by allowing them to feed on pledgets soaked with a mixture of HP virus, blood, sucrose, and serum were unsuccessful. The amount of virus available in the artificial blood meals was less than 100 pfu which may have been below the

infectivity threshold. Other mosquito-borne viruses have a much lower efficiency of infection by the oral route than if inoculated parenterally (Hardy et al, 1983). This also appears to occur for HP virus. The low titer of available viral stocks and inability to produce viremia in a vertebrate host hindered any further investigation of oral infection of Cx. tarsalis with HP virus.

Attempts at transovarial transmission.

There was no evidence of TOT of either TUR or HP virus in Cx. tarsalis. The use of a high pH buffer (BABS) instead of the standard mosquito diluent to overcome the detrimental effect of normal larval tissues (Ksiazek et al, 1983) did not change the findings. The numbers of progeny evaluated were large enough that TOT would have been detected if there were nothing inherently abnormal about the viruses or the mosquitoes used in these studies. TOT certainly did not occur at the rates which have been demonstrated for other viruses in which TOT apparently plays an important role in viral maintenance (Tesh, 1980; Turell et al, 1982b; Miller et al, 1982). It is unlikely that TOT is responsible for a significant portion of the TUR or HP viral infection rates observed annually in California (Easons series). However, it is still possible that there are "clones" of mosquitoes which transmit the viruses at very high percentages as has been demonstrated for California encephalitis and San Angelo viruses (Tesh, 1980; Turell et al, 1982b) and that such clones are much rarer

for Cx. tarsalis and TUR and HP viruses than were found in the above studies. Another possibility is that the combination of viruses and mosquitoes employed in these studies was unsuitable for the establishment of such a relationship. Investigations with sigma virus have shown that the genetics of both the virus and the insect play a role in viral replication in a particular virus-insect combination (Brun and Plus, 1980).

Alternative possibilities for maintenance of arboviruses have been reviewed by Reeves (1974) and some of these alternatives do not necessarily directly involve the apparent summertime mosquito vector. TUR virus has been shown to infect house finches persistently although the virus was not recovered from Cx. tarsalis fed on such birds (unpublished). This possibility was not evaluated for HP virus in the current studies.

#### CO<sub>2</sub> sensitivity

The lack of CO<sub>2</sub> sensitivity induction by HP virus was somewhat unexpected. Several other rhabdoviruses, including Gray Lodge and FLA, which are antigenically related to HP virus, have been shown to induce CO<sub>2</sub> sensitivity in mosquitoes (Rosen, 1980). The methods used to evaluate sensitivity (Rosen and Shroyer, 1981) were more rigorous than those previously employed in the AVRU laboratories (Turell and Hardy, 1980; Turell et al, 1982b) but CO<sub>2</sub> sensitivity still could not be demonstrated. The negative findings with TUR virus reaffirmed

previous findings (Turell and Hardy, 1980; Turell et al, 1982b). The efficacy of the methods used was confirmed by induction of sensitivity to CO in Cx. tarsalis infected with VSV.

2

#### Vertebrate infection

The failure of chickens to become infected with HP virus following parenteral inoculation was not unexpected because previous results from tests in other laboratory hosts had been negative (unpublished). It was thought that resorting to infection of 1 day old chicks by the intracranial route would result in evidence of infection as had been shown by Whitney for FLA virus (Whitney, 1964) but neither HP (BFN5662) nor HP (AR70) produced antibody responses at 21 days post-inoculation. Serological findings in sentinel flocks are supportive of these findings (see Chapter III). No chickens developed IFA titers in flocks in which large proportions of the chickens had acquired antibody to both TUR and WEE viruses and mosquitoes in the area were infected with HP virus. The ubiquitous distribution of HP viral infections in Cx. tarsalis leads one to assume that infected mosquitoes were feeding on these same chickens. Results of transmission experiments to droplets would indicate that Cx. tarsalis could have transmitted at least small amounts of HP virus into these sentinel chickens. It would seem, therefore, that chickens must be refractory to infection with HP virus (at least to the BFN5662 and AR70 strains).

III. ANTIBODIES TO  
TURLOCK AND HART PARK VIRUSES IN THE SERA OF SELECTED VERTEBRATES  
IN CALIFORNIA.

INTRODUCTION

The primary aim of this study, beyond the development of a serologic test system, was to determine which species of animals were being exposed to TUR and HP viruses. Paired sera collected from persons and horses with CNS disease were tested to determine if these viruses were causally related to disease. A number of different serum "banks" accumulated over the years by a cooperative effort of the AVRU and the VRDL were also tested to determine the prevalence of past infection with TUR or HP viruses.

A secondary aim of the study was to evaluate and use the IFA test for the detection of antibodies to TUR and HP viruses. PRN tests were performed on samples of sera tested for HP and TUR virus to permit comparison with the IFA tests.

Determination of the role of any virus as a cause of disease depends on the ability to isolate the virus from animals during the acute stages of infection or disease and on the detection of antibodies as an indication of current or past infection. The latter usually is determined by 1 or more serological procedures to detect specific antibodies for the virus of interest. Such data provide

information about the range of host species infected by the virus, the age when animals were infected and the geographical distribution of the agent.

A number of standard serologic procedures have been used to detect arboviral antibodies. Most prominent among these probably has been the HI test (Clark and Casals, 1958). This test has proved very useful to detect a wide range of arboviral infections. However, some groups of arboviruses, including the Rhabdoviridae of which HP virus is a member, do not produce detectable hemagglutination of erythrocytes from a range of species. TUR virus produces a hemagglutinin but as with many other viruses in the family Bunyaviridae, hemagglutination titers are relatively low as compared with the traditional alpha- and flaviviruses (Beaty et al, 1977). In previous studies in the AVRU laboratory, it was found that the HI test for TUR virus did not detect antibodies as well as the Nt test in the sera of a variety of animals (unpublished). The Nt test probably is the best available test as an overall serological procedure. It has proven to be sensitive, the antibody it detects is long lasting, and in the sera of most species for most viruses it is the most specific test available.

#### MATERIALS AND METHODS

IFA tests.

A micro adaptation of the IFA test was employed following a previously described method (Gallo et al, 1981). The only major variation was that the infected cells were frozen and many lots of slides were prepared from identical aliquots of frozen viral antigen bearing cells.

Monolayers of the 0853 line of hamster kidney cells grown in 16 oz prescription bottles were infected with an appropriate dilution of the required virus. The majority of cells contained detectable viral antigens within 48 to 72 hours. The cells were scraped from the growth vessels and combined with uninfected cells to yield approximately 1/3 antigen positive cells and 2/3 antigen negative cells. The cells were washed once with PBS (Dulbecco and Vogt, 1954) and pelleted by centrifugation at 200 X G. The cells were then trypsinized for 2 minutes in a trypsin and ethyldiamine-tetraacetic acid solution (Lennette and Schmidt, 1969) and subsequently washed twice in PBS. Cells were then resuspended for freezing in a medium of 10% dimethyl-sulfoxide in Eagles' MEM with 5% FBS and 0.35 g/l NaHCO<sub>3</sub>. The cell suspension was adjusted to contain a sufficient number of cells so that a drop placed on a slide appeared to be nearly confluent when viewed through an inverted microscope at 100 X magnification. In general, three 16 oz prescription bottles (1 infected and 2 uninfected) yielded 10 ml of such a cell suspension. This suspension was dispensed in 0.5 ml aliquots in conical polypropylene vials and slowly frozen in an insulated container at -70 C.



Slides were prepared by fast thawing the frozen cell suspension in a 37 C waterbath. Cells were washed in 0.01 M PBS, pH 7.2 (PBS-FA) and centrifuged at 200 X G. This was repeated and then the cells from each tube were resuspended to a final volume of 0.1 ml of PBS-FA. Slides coated with Teflon leaving 12 wells of approximately 6 mm in diameter were used for all procedures. Micro-spots were placed on the slides with a simple spotting device made from a blunted 26 gauge needle attached to a Caraway tube. Care was taken to mix the cells to remove any clumps in the cell suspension. The cells in the dispenser were replaced with fresh cells from the suspension after dispensing approximately 150 spots. Six micro-spots infected with separate viruses were placed in each well of the slides. Thus, 12 sera could be screened against 6 different antigens on 1 slide. Slides were air dried, fixed in chilled acetone for 10 minutes and then stored at  $\leq$  -70 C until used.

Sera to be screened for antibody were diluted 1:8 in a suspension of 20% beef brain in PBS-FA. The sera of ungulates were diluted 1:8 in a suspension of 10% beef brain and 10% homogenized tissues of skinned and eviscerated hamsters in PBS-FA. Sera were adsorbed in these diluents overnight at 4 C. A single drop of the diluted serum to be tested was placed in each slide well by capillary pipette. Diluted sera were incubated on the antigen spots for 30 mins in a humid chamber at 37 C. Sera were flushed off of the slide surface using a gentle stream of PBS-FA from a gravity fed dispensing bottle. This minimized the possibility that wells would be cross-contaminated with high titered antibody from adjacent wells. Slides were then washed

twice for 10 minutes with PBS-FA. One of the advantages of the use of the pre-drawn pattern of spots on the slide was the conservation of reagents. Six slides were placed in a row on top of a paper towel and then covered with another paper towel to blot them dry. Gentle pressure was then applied to the top paper towel. This procedure dried the Teflon spaces between the wells on the slides and prevented the conjugate, when applied, from running over the entire surface of the slide. Thus, the amount of conjugate necessary to flood the wells was minimized. The anti-immunoglobulin conjugate, diluted in PBS-FA, was allowed to incubate with the serum reacted antigen spots for 20 minutes. The slides were then washed twice for 10 minutes in PBS-FA. Cover slips were mounted on the slides using glycerol to which 1/10th volume of PBS-FA had been added.

Except for tests on human sera, commercial anti-IgG conjugates were used for the species of interest. Human sera were tested with the use of a combination of anti-IgG, anti-IgM, and anti-IgA conjugates. All sera had been conjugated to FITC and was obtained from Cappel Laboratories.

The proper dilutions of anti-globulin FITC-conjugates were determined by titration of the conjugate against dilutions of known positive sera. In some instances screening of a number of sera was performed with a 1:20 dilution of the FITC-conjugate until a positive for that species was encountered and then a titration was performed of the FITC-conjugate. The FITC-conjugates used in these studies were of very high quality. The FITC-conjugates themselves left no background fluorescence when reacted with the antigen containing cells. The

FITC-conjugates all were used at dilutions of from 1:40 to 1:80.

Neutralization tests.

PRN tests for HP and TUR viruses were performed after a previously described method. (DeMadrid and Porterfield, 1969). The procedure was modified as follows: TUR tests utilized a 1:250 suspension of the cell pellet of primary duck embryo cells; HP tests used Vero cells and an overlay of 0.75% methyl cellulose instead of 1.5% carboxymethyl cellulose. Sera were heat inactivated at 56 C for 30 minutes and tested at a dilution of 1:10 in PBS containing 0.5% gelatin. Cell monolayers in the TUR viral tests were fixed after 3 or 4 days while the cell sheets in HP viral tests were fixed after 6 or 7 days. Cells were fixed overnight by adding formalin U.S.P. to each well of the plate. The cells were stained the following day with 0.5% crystal violet in 10% formalin. The criterion for neutralization was an 80% reduction of a viral test dose that represented from 25 to 100 plaque forming units (PFU). Chicken sera on which PRN tests had been performed previously for TUK, SLE, or WEE had been tested by another method (Earley et al, 1967) but used the same criterion for neutralization.

PRN rather than IFA tests were employed for a number of wild animal species due to a lack of commercially prepared FITC-conjugated antisera. Sera from wild carnivores were obtained from either the

Vector Biology and Control Division (VBCD) of the CDHS or from the Plague Branch, Centers for Disease Control. These were whole blood specimens dried onto paper filter strips. The filter strips were eluted into 2.0 ml of PBS with 0.5% gelatin and heat inactivated at 56 C for 30 minutes. The sera were then centrifuged at approximately 200 X G for 10 minutes and the supernatant fluids were used in the PRN test. It was assumed that this represented a nominal dilution of 1:10 although it may have been slightly higher. Frozen sera from wild avian and mammalian species were available as a 1:5 dilution. These sera were inactivated at 56 C for 30 mins and then brought to a final dilution of 1:20 in PBS with 0.5% gelatin.

#### Viruses.

The 847-32 strain of TUR virus was used for both the IFA and the PRN tests. The virus had received 1 passage in chick embryos and 10 subsequent intracranial passages in suckling mice. The stock virus consisted of a 10% suspension of infected mouse brain.

The BFN 5662 strain of HP virus was used for the IFA and PRN tests. Virus for IFA tests had received 2 passages in Vero cells, 2 intracranial passages in suckling mice, and 6 further passages in Vero cells. PRN tests were performed with a Vero cell adapted strain in its 27th Vero cell passage. The BFN 5662 strain was used because of difficulty in obtaining antigen infected cells or consistent plaquing with the prototype AR70 strain of HP virus.

Four additional viruses were included in the serological tests to validate the test and to explore the host range of these viruses. The viruses utilized were WEE, BFS1703, smb2; SLE, BFS1750, smb2; Llano Seco (LLS), BFN3112, DECC8, C6/36-1; and Gray Lodge (GRL), BFN3187, Vero 7. Viral stocks were maintained at -70 C.

#### Sample populations.

The populations tested for antibody are described in Table III-2. There were different objectives in testing the various populations. Populations with recent histories of CNS disease were tested as a retrospective study for a possible association of HP or TUR virus with disease. Tests on sera from these populations had an additional value in that they provided a crude, albeit biased, estimate of the prevalence of infection with these viruses. Sera from sentinel chickens were tested to determine the rate at which the viruses were being transmitted within geographical areas over time. Sera from cross-sectional population surveys of a range of animal species were tested to determine the past prevalence of viral infection.

Table III-3 lists the geographical distribution by county of residence of the human cases of CNS disease that contributed paired sera tested for IFA test antibody. Tables III-4 and III-5 list the counties of residence of the horses contributing diagnostic pairs and domestic mammal cross-sectional populations sampled, respectively. Table III-6 lists the counties from which the wild mammal samples were

taken. The geographical distribution of sentinel chickens which were tested for antibodies is given in Table III-7.

## RESULTS

### Validation of the IFA test.

Hyperimmune mouse ascitic fluids (HMAFs) to 17 arboviruses, known to occur in California (Hardy, 1970), were used to evaluate the cross-reaction pattern of TUR and HP along with the 4 other IFA antigens used routinely in the study (Table III-1). HP and TUR did not cross-react with the other viruses. As expected, SLE antigen cross-reacted with the HMAFs of the other flaviviruses (Rio Bravo, Modoc, and Powassan) that occur in California. LLS antigen did not cross-react with Bluetongue type 8 virus. GRL antigen did not cross-react with antibodies to either HP or Kern Canyon, the other 2 rhabdoviruses known to occur in the state. HP antigen cross-reacted with antibody to Flanders virus, a closely related virus which occurs in the Eastern and Central United States (data not shown). Therefore, the IFA test procedure appeared to be specific with the exception of SLE for the viruses of interest.

Tests on convalescent diagnostic serum specimens  
from human CNS cases.

IFA tests were performed on the convalescent sample of 1,732 paired sera from human CNS cases. One reacted to TUR and when the acute and convalescent sera were tested together had a stable titer by both IFA (1:128) and PRN test (1:80). None of the 1,732 sera reacted to HP virus.

PRN tests were performed on 236 convalescent sera obtained between 1979 and 1981. None of the sera that were negative in the IFA test neutralized 80% of the test dose of TUR virus. However, 13 of 236 convalescent sera negative for HP IFA antibody neutralized 80% or more of the test dose of HP virus when tested at a 1:10 dilution (Table III-8). These sera were then retested with the acute phase sample against a similar dose of virus with 2-fold serial dilutions of the sera (Table III-9). A number of the paired sera neutralized the virus and in 5 pairs there was a rise in titer between acute and convalescent samples. However, none of the sera had titers higher than 1:160.

HI tests for TUR virus had been done earlier on the samples from 1968 to 1976. Table III-10 compares the results of these HI tests to the current IFA tests. The serum that was positive by IFA test and confirmed by PRN was not positive in the HI test. The sera that reacted in the HI test did not react in the IFA test and was not tested by PRN.

The sera tested for HP and TUR viral antibodies also were tested

for WEE and SLE IFA antibodies for 2 reasons: first, to compare the results of the micro-dot IFA test against those of previous HI tests; secondly, to determine if the sera used in these tests, some of which had been in storage for 15 years, had deteriorated to the point where antibody for any virus would not be detectable. Comparisons for WEE virus (Table III-11) and SLE virus (Table III-12) showed that the majority of the HI positives were detected by the IFA test for both viruses. It may be that some of the HI positives were in fact non-specific reactors, a well known drawback of the HI test (Shortridge, 1977) against which special precautions must be taken (Clark and Casals, 1958; Monath et al, 1970).

Tests on the convalescent sample of diagnostic sera  
from CNS cases in horses.

Tests on 146 pairs of sera taken from horses with CNS disease are presented in Table III-13. Eight horses had IFA antibody for TUR virus in the convalescent serum sample; however, when the acute and convalescent sample were examined together, none of the pairs had a titer rise. Rising titers for TUR virus were expected in some of these cases since horse cases of CNS disease had been associated with TUR infection previously by serological means (Hardy, 1970). Three of the horses showed evidence of HP infection on IFA tests; however, IFA tests revealed very low standing titers in both acute and convalescent samples. PRN tests were also done on 97 of the convalescent sera.



There were 7 sera that reacted by HP PRN test but did not react in the IFA test while there were no sera which reacted by both the HP PRN and IFA test. Four of the 7 PRN test positive convalescent sera were tested with their acute sample and all had low (<1:40) titers in both acute and convalescent samples. The sera of 14 of the 97 horses tested for TUR PRN antibodies reacted at greater than 1:10. Four of these also had IFA antibody. One sera was positive by IFA alone. TUR PRN test titrations were not done on the PRN screen test positive samples.

Serological tests on cross-sectional human population samples, Kern County, 1960.

Serum samples had been taken from people resident in rural Kern County in 1960. This population was tested by IFA test for the 6 antigens of interest. No sera reacted at a 1:8 dilution with either HP or TUR antigens. However, the reaction rate was 86/235 (37%) for SLE and 32/235 (14%) for WEE virus. Thus, these samples were taken at a time when many more people were being infected with WEE and SLE viruses than has occurred in recent years. Comparison of the current data with those of previous HI and mouse Nt tests (Froeschle and Reeves, 1965) on these same sera indicated that the IFA test did not detect a number of positives. Of sera that were tested by IFA test, HI, and mouse neutralization tests; 18/157, 20/157, and 53/157, respectively, had antibody for WEE virus. Of the sera tested for SLE

virus antibody by all 3 methods 59/157, 50/157, and 72/157 were positive by the IFA, HI, and mouse Nt tests, respectively.

Serological tests on cross-sectional domestic mammal samples

IFA tests for TUR and HP viral antibodies in a sample of domestic mammals are summarized in Table III-14. Only horses, dogs, and pigs had appreciable proportions with antibodies for TUR virus, 25%, 30%, and 8% prevalence, respectively. Very few domestic animals other than dogs had detectable HP viral antibody, 2/497 (<1%) horses, and 16/44 (36%) dogs.

A sample of 100 horses, 50 cattle, and 50 sheep sera and all available dog, swine, and goat sera were tested by PRN for HP antibodies (Table III-15). The IFA test for HP viral antibody may have missed a considerable number of animals with neutralizing antibody.

Results of the HI and IFA tests for TUR antibodies are presented for each individual species in the domestic mammal population in Table III-16. PRN tests for TUR antibodies on these same sera resulted in 293 valid tests. The results of HI and IFA tests on sera for which the PRN test had also been done are summarized in Table III-17. These data indicate that when the PRN test is known to be negative for TUR antibody, an approximately equal number of sera give "false" positive reactions by both the IFA test and the HI test. If only the PRN positive stratum is considered, the IFA test seems to fare slightly

better (22 sera detected by IFA and missed by the HI test) than does the HI test (12 sera detected by the HI test while missed by the IFA test) for detection of TUR antibody. However, neither the HI nor the IFA test did well when compared with the PRN. The HI test detected only 48/87 of the TUR PRN test positive sera in the sample of 293 animal sera tested. The IFA test detected 58/87 of the "true" positives.

The detection of WEE and SLE antibody also was used to indicate how well the several tests had detected antibody. The HI test detected 475/1,544 animal sera with WEE antibody while the IFA test detected 355/1,544 (Table III-18). Both tests agreed on 335 of these sera. The IFA test had 20 "false positives" according to the HI test. Conversely, the HI test had 137 "false positives" according to the IFA test. The findings in a comparison of the IFA test and HI tests for SLE virus were very similar (Table III-19). The HI test detected 239/1544 SLE reactors while the IFA test indicated that there were 120/1544 positives among all the species in the sample. Again there were a number of "false positives" in both directions of detection. The IFA test had 36 such reactors while the HI had 148 such reactors. The tests co-detected 84 with SLE antibodies from among the sample. No PRN tests were performed for either SLE or WEE viruses so it is difficult to determine which of the 2 tests was a better indicator of the true antibody status of the animals.

Non-specific IFA reactors were common in the sera of ungulates. This was particularly true for the sheep and cattle. Those horses which had non-specific staining of antigen containing cells had a

lower level of background than the cattle or sheep and a lower proportion of animals with the problem was encountered. The sera of all 3 of these species were adsorbed with hamster tissues and this decreased but did not alleviate the problem entirely. Another possible solution to the problem of non-specific reactions would have been to dilute the sera further. However, titration of sera from dogs, horses, and swine in the cross-sectional sample indicated that TUR antibody titers in the positive samples were not high (Table III-20). This probably reflected either a low level of antibody response, a short duration, or both of TUR antibody in these species.

Dogs were the only species that had a relatively high prevalence of HP viral IFA antibodies. The titer of antibodies in the sera of positive dogs generally were high, ranging from 1:64 to  $\geq$ 1:256.

#### Additional cross-sectional samples of mammals

The relatively high prevalence of HP and TUR antibodies in dogs led to an effort to obtain sera from feral carnivores and additional domestic dogs. None of the sera from wild carnivores neutralized either HP or TUR virus (Table III-21). However, an examination of the areas (Table III-6) from which these specimens were collected indicated that most were from areas in California where Cx. tarsalis is not prevalent. Therefore, it is not surprising that there were no positives among these sera.

Sera also were collected from military working dogs living in the

Central Valley of California and pet dogs from Bakersfield, California to determine if the prevalence of HP antibodies had remained relatively high in this species (Table III-22). PRN antibody prevalence in the sera of these dogs was not at the same high level found in the 1968 cross-sectional sample of dogs. The sample of dogs from Bakersfield came largely from dogs which were classified as living in urban areas. The oldest dog in the sample was 7 years old, thus indicating that transmission to dogs has occurred in the last several years.

Results of PRN tests on sera from a variety of rodents and lagomorphs collected in Kern County between 1969 and 1971 are summarized in Table III-23. This sample was drawn at random from mammalian sera collected during the above time and location and should be representative of the species collected. (The sample is not necessarily representative of the animals living in the area.) Antelope ground squirrels and the kangaroo rat were the only species with one or more than one positives to either HP or TUR virus by PRN test. None of the jackrabbits or cottontail rabbits were positive for either virus.

#### Wild birds

Tests on sera from wild birds collected in Kern County indicated that the house finch had a higher prevalence of PRN antibodies for HP (53/76) and TUR (33/65) than any of the other animal species tested

(Table III-24). The lack of either HP or TUR PRN antibody in house sparrows possibly reflected that the 2 species were not collected in the same location. However, 6 of the sparrows were collected at the location where the majority of the house finches were collected and none of these 6 birds had PRN antibody for either HP or TUR viruses. The birds included in this sample were either adult birds or juveniles collected late in the summer.

#### Sentinel chickens.

Sentinel chicken flocks are maintained each year in locations throughout the state as part of an overall arboviral surveillance effort. The flocks are bled monthly from the beginning of May until the end of September. These sera offered an opportunity to follow the transmission pattern of a virus within the locations where the flocks were maintained. Sentinel chicken flocks had been tested for TUR PRN antibodies previously in 1978 and 1979 and the results of tests on the final samples of the year were compared with the results of IFA tests for TUR viral antibodies (Table III-25). The IFA test was much less sensitive for the detection of TUR viral antibodies if the PRN test reflected the true antibody status of the chickens. The relative sensitivity of the IFA test was 83.5% for the 2 years in which both tests were performed on a total of 1070 chicken sera. There appeared to be few false positives as only 18 sera were positive in the IFA test that did not react in the PRN test, yielding a relative

specificity of 97.6%. The titer of antibodies in the sera of 89 chickens which screened positive in the IFA test was determined and 77/89 had titers of 1:64 or higher. It is not known why so many chickens which were classified as positive by the PRN test were negative in IFA tests.

A comparison also was made between the IFA and the PRN tests for both SLE (Table III-26) and WEE (Table III-27). SLE virus was at a low ebb during 1978 and 1979 so there were few sera upon which to make a comparison. However, there seemed to be a tendency to classify quite a few sera as IFA positive which did not neutralize SLE virus. This has been observed by others in their initial evaluation of the standard IFA test (Emmons et al, 1980). The comparison of the IFA to the PRN test for WEE virus was much more favorable. The relative sensitivity of the IFA to that of the PRN test was 97.0% while the relative specificity was 99.3%. Another set of comparisons was made between the standard IFA test and the micro-IFA test employed in these studies. SLE viral antibodies have not had a high prevalence in the chicken flocks over the last 5 years with only a total of 19 birds available for testing which had been recorded as having been positive in standard IFA tests (Table III-28). The micro-IFA test detected 14 of these 19 sera as positive while detecting an additional 14 chickens as positive which were classified as negative by the standard IFA test. This yielded a relative sensitivity of 73.7% and a relative specificity of 99.4%. A comparison of the response of the standard IFA test and the micro-IFA tests was much more favorable for WEE antibodies (Table III-29). The relative sensitivity of the micro-IFA

test to the standard test was 97.5% while the relative specificity was 98.6%.

## DISCUSSION

### Evaluation of the IFA test

Evaluations of the use of the IFA test have given mixed results. Clearly the test is not as effective as the PRN test to detect past infections with TUR virus. The best results were from chicken flocks where infections had occurred shortly before serum collection--certainly no greater than a few months before the last sera of the year were collected. In the sentinel chickens approximately 83% of the PRN positive animals were detected by the IFA test. This could be an indication that the IFA test is not very sensitive for detecting the reaction of a chicken to infection with TUR virus or that the PRN test possibly was in error. The IFA test would seem to be sensitive in terms of the titer of antibody in birds which were infected because 89 birds which were determined to be IFA positive had titers in excess of 1:64. Some chickens may have a very poor IFA response to infection with TUR virus. No sera were available from experimentally infected birds to test the time course of TUR IFA antibody response. Others (Scott et al, 1983) found that some gallinaceous birds did not respond with neutralizing antibody



following experimental infection. For instance, 0 of 15 Japanese quail responded to inoculation with almost  $5 \log_{10}$  of TUR (847-32) virus. The same authors found that 7 of 7 chickens inoculated with the same dose of virus developed PRN antibody. However, the chickens were only 1 day old when inoculated and there may be an age-susceptibility barrier to infection and antibody response.

Another problem is to determine the duration of detectable levels of antibody once infection does occur. The cross-sectional samples of domestic and feral animals were not as likely to have been infected recently as were the chickens. The sentinel flocks were replenished each spring so infected birds had to have been infected during the current year. The cross-sectional sampling of populations used would have included individuals infected several years in the past and protected from any further infections by that experience. The IFA test for LaCrosse viral antibodies was very suitable for the diagnosis of recent human infections but was not effective for detection of more distantly occurring infections (Beaty et al, 1982). LaCrosse virus is now classified in the Bunyavirus genus as is TUR virus (Klimas et al, 1981). There is a possibility that a parallel may exist in the serological response of some species to these 2 viruses.

A further obstacle to evaluation of the sensitivity of the IFA test was the relatively low prevalence of infection in the majority of species which were tested in the cross-sectional surveys. Since the purpose of this study was to evaluate "viruses in search of a disease" there were no proven cases with which to make comparisons.

Evaluation of the efficacy of the IFA test to detect past

infection with HP virus was even more difficult than for TUR virus. Even though the virus was isolated from Cx. tarsalis at similar or higher rates than TUR virus, fewer infections seemed to be occurring in most animal species.

Comparison of the micro-IFA used in these studies to those of the standard macro-version employed by the VRDL was quite favorable for WEE antibodies. However, there was a much poorer agreement between the 2 tests for detection of SLE antibodies. One possible explanation of these discrepancies is that the standard IFA test was performed at dilution of 1:40 while the micro-IFA test was performed at a dilution of 1:8. The 1:40 dilution used in the standard IFA test was selected after considerable trial and error to avoid the misclassification of sera as positive. The serum dilution selected for the micro-IFA test was set low intentionally because the response of individual species, including chickens, to viruses other than WEE and SLE was unknown and the detection of such antibodies was of primary concern. Another possible source of discrepancies between these tests was clerical errors or mislabelling of specimens in the course of collection, recording, and analysis of the data. Large numbers of individual observations were involved and occasional anomalies such as chickens that seroconverted and then reverted to negative status were observed (unpublished). When the volume of data is so large it is difficult to resolve such matters by retesting or completely reviewing all data.

The IFA test as a whole was relatively easy to perform and for certain purposes seemed to detect the majority of past infections of several species with WEE, SLE, or TUR viruses. Because antibody was

rare in all species but the dog (tested by more than one serological test), evaluation of the efficiency of the IFA test for HP antibody was difficult. The use of a micro-spot protocol allowed screening sera for antibodies to multiple antigens in a single well of a microscope slide. The test was economical in terms of both effort and cost of reagents. The use of a pre-frozen suspension of infected cells as the antigen substrate in the test insured that the quality of the antigen preparation remained constant from batch to batch of slides. The freezing of antigen bearing cell suspensions also facilitated the "custom making" of slides with required combinations of antigens for a particular purpose. One obvious deficiency of the test was a requirement for available FITC-conjugates for all species to be tested. This deficiency required that the sera of many wild animal species be subjected to the PRN test.

#### Serological findings

It is best to recall the possibly biased method of collection of the cross-sectional samples used in these studies. These were convenience samples of the total or target population. For this reason, the antibody prevalence should be considered a crude and possibly biased estimate of the overall prevalence. However, because population based data for planning a random sample are not readily available and because the expense of collecting such a sample is great, the use of previously existing serum banks can be viewed as a

necessity.

The results of tests on the human and equine sera collected from individuals with recent history of CNS disease indicated that neither of these viruses was an important cause of such disease. There were 5 pairs of human sera with rising titers (4-fold or greater) by HP PRN test. These sera were of relatively low titer (not more than 1:160) and repeated PRN tests on the sera gave consistent low titers while the IFA test results were negative. Caution should be exercised in accepting the results of even the preferred Nt test as others have found that the sera of some animal species contain non-specific substances which neutralize virus (Scherer, et al, 1972). The low prevalence of either PRN or IFA antibody to HP virus in either the human or equine cases indicates that there was little chance of these viruses having been involved as causes of other diseases unless they are fatal or in some other way reduced the probability an individual would enter the sample. This can also be said of TUR virus infection in man. However, TUR viral infections appeared to be common in horses. The lack of association of CNS disease with recent TUR infection probably indicated that TUR virus was not a major cause of CNS disease in horses. However, an occasional case may occur and other syndromes may be associated with TUR viral infection of horses.

There were no positive IFA tests for HP viral antibody in chicken sera. This would seem to confirm their lack of susceptibility as chickens inoculated with the virus did not produce IFA or PRN antibodies (Chapter II). FLA neutralizing antibodies were not detected in the sera of sentinel chickens in areas of active FLA viral

transmission in Illinois (Kokernot et al, 1969). Horses had 2 HP IFA positive sera in the cross-sectional survey population. Dogs had the highest prevalence of IFA antibody (16/44) of any of the mammals tested from collections made in 1968. Subsequent samples drawn from dogs in 1983 also had antibody prevalence (10/77) higher than any of the other mammal species tested. The relatively high infection rate of dogs with HP virus possibly is worth further investigation as this species could suffer disease as a consequence of infection with the virus. The type of samples tested during this study did not allow any evaluation of this possibility. A high antibody prevalence in dogs might result if previous vaccination against rabies virus potentiated the antibody response to infection with HP virus. It seems unlikely that vaccination with rabies virus alone would lead to either IFA or PRN test antibodies to HP virus. The lack of relationship of HP virus to the rabies serogroup of viruses has been established (Berge, 1975; Shope, 1975). Sera from 2 humans who had been repeatedly vaccinated with rabies virus, including the potent WI-38 vaccine, did not react with HP virus in current IFA tests. Military working dogs are vaccinated annually against rabies virus yet very few reacted with HP indicating that the IFA test with HP did not detect rabies vaccination induced antibodies.

House finches had a high prevalence of PRN antibodies to both HP and TUR viruses. House finches previously were the major species of bird found to have HI antibody to TUR virus, 33% prevalence (unpublished). In the present studies the prevalence of PRN antibody in house finches collected in Kern County was 33/65 (50.8%) for TUR

virus and 53/76 (69.7%) for HP virus. House sparrows collected during the same time did not have antibody to either virus although some of the birds were collected from the same location. The finding of HP antibodies in house finches might be expected since the virus was isolated from this species by Johnson (Berge, 1975). However, FLA was isolated from several species of birds in the Mississippi/Ohio River Basin but limited serological surveys of these same species of birds failed to find more than an occasional "equivocal" reaction by the mouse Nt test (Kokernot et al, 1969). The sera of 130 sentinel chickens, exposed through the transmission season in areas where FLA virus was isolated repeatedly, and 59 human residents all were negative when tested for FLA viral Nt antibody (Kokernot et al, 1969).

The above findings indicate that HP viral infection does occur in mammalian species but the rate of infection appears to be very low with the possible exception of dogs. The number of dogs in an area and their rather long lifespan would indicate that this species is capable of playing no more than a minor role, if any, in the maintenance of the virus.

The finding of antibody to both HP and TUR viruses in the sera of house finches indicates that these birds may in fact be playing a role in the amplification of the viruses. This species is plentiful in areas where the apparent vector, Cx. tarsalis is found (Reeves and Hammon, 1962). Cx. tarsalis has been found to feed with great regularity on these birds. Analysis of 4,220 Cx. tarsalis which had fed upon passerine birds in Kern County between 1960 and 1965 demonstrated that 30.4% of the birds that had taken blood meals from

passerine birds had fed upon the house finch (unpublished). Previous reports had indicated that 46.4% of all Cx. tarsalis collected in Kern County over a 3 year period from 1960 to 1963 fed on passerine birds (Tempelis et al, 1965). Isolations of FLA virus were made only after sampling emphasis was shifted to nestling birds (Kokernot et al, 1969) and this may indicate that there is an age restriction on the replication of this group of viruses in birds and that isolations of the virus from mosquitoes may parallel the nestling season in birds. House sparrows and house finches nest in Kern County during the months of March to August and the bulk of nesting activity is between April and July (Reeves and Hammon, 1962) a period during which HP viral isolations seem to peak. Further investigations of the house finch as a vertebrate host of TUR and HP viruses is warranted. It should be proven that neutralizing substances found in the serum of these species is specific antibody and the duration and magnitude of HP viremia in house finches should be determined. It would also be worthwhile to determine the effect of age on the ability of house finches to produce viremia and respond with antibody to HP virus.

IV. ANALYSES OF TURLOCK, HART PARK, AND WESTERN EQUINE  
ENCEPHALOMYELITIS VIRUS PERSISTENCE IN CULEX TARSALIS IN CALIFORNIA.

INTRODUCTION

The persistence of HP and TUR viruses in California over the last 5 years is well documented (Emmons series). HP and TUR viruses seem to be different in their pattern of persistence from the 2 arthropod-borne viruses of primary public health concern in California, western equine encephalomyelitis WEE and SLE. Therefore, an examination was undertaken of the dependence of TUR, HP, and WEE viruses upon variables known to influence the prevalence of many arthropod-borne viruses (Reeves, 1967). These analyses were confined to Cx. tarsalis because approximately 90% of all mosquitoes tested were of this species and very few isolates of any of these viruses were obtained from other species of mosquito in California. SLE virus was not included in these comparisons because activity of this virus was confined largely to the southeastern portion of California and was very sporadic in its occurrence in even this limited area.

Reeves (Reeves, 1967) outlined many of the important variables which are involved in the maintenance and propagation of an arbovirus. Clearly, the study of an arbovirus is complicated and requires a coordinated analysis of these interrelated variables. The analyses performed in the present effort were limited not by a lack of recognition of other important parameters but by the lack of access to



data.

## MATERIALS AND METHODS

### Virus isolations.

Data on numbers of mosquitoes tested and number of viral isolations were obtained from the VBCB and the VRDL. Yearly summaries of viral isolations have been reported previously (Emmons series). The current analysis is limited to the period 1978 to 1982. These years were selected because the viral isolation methods (inoculation of suckling mice) remained constant throughout this 5 year period. Data that were computerized included the species of mosquito; the date, method, and county of collection; the number of individual female mosquitoes included in the pool; and the results of the isolation attempt. Data were organized so that the number of Cx. tarsalis collected and tested and the number of viral isolates from mosquitoes were available for month-county units throughout the 5 year period. Timing of the mosquito collections precluded stratification of data into shorter time intervals. Viral isolations from other mosquito species as well as those from Cx. tarsalis have been reported previously (Emmons series). Only 5 isolates of HP and 3 of TUR were made from other mosquito species so the present study was limited to isolates from Cx. tarsalis the apparent primary vector.

The above organization of data allowed the calculation of a MIR for each virus for each month-county unit. The MIR used in these analyses was the number of isolates of a particular virus divided by the total number of Cx. tarsalis tested multiplied by 1000. The rate is referred to as minimal because isolation of virus from a pool of mosquitoes only indicates that at least 1 infected individual was present in the pool (Chiang and Reeves, 1962). The vast majority of mosquito pools contained 50 individuals.

#### Mosquito light trap indices.

A standard light trap index (LTI) was used as a measure of the relative abundance of Cx. tarsalis. This index was the number of females collected per New Jersey light trap per night. Weekly records of the arithmetic mean of the number of female Cx. tarsalis collected in both urban and suburban-rural locations had been accumulated by the AVRU. There is more than 1 mosquito abatement district (MAD) located in several counties in California. There also were 3 MADs that incorporated two counties (Sutter-Yuba, Sacramento-Yolo, and Marin-Sonoma). Weekly MAD light trap records were combined into a monthly summary figure for the county. Both urban and suburban-rural light trap indices were individually combined using a logarithmic conversion ( $\log [LTI+1]$ ) and a mean was calculated for the month-county unit. The month-county mean LTI was adjusted for the total number of valid weekly reports from each of the respective MADs

within the county.

Sentinel chicken serologies.

Sentinel chickens have been maintained for a number of summers in representative areas of the State as a primary surveillance technique to detect viral transmission (Reeves and Milby, 1980). Serologic evidence of past infection by WEE and SLE viruses for this 5 year period was reported by Emmons and associates (Emmons series). The IFA test was used by the VRDL for the years 1979 to 1982 to detect WEE and SLE antibodies. The results of these tests were available within the AVRU and were used to determine the seroconversion rates. Comparable data on WEE had been obtained for 1978 from neutralization tests performed by the AVRU laboratories.

In the present study sera from the above birds for the years 1980-1982 were tested for TUR antibody by the IFA test. TUR PRN test data were used for the years 1978 and 1979. Comparison of the results of TUR IFA and PRN tests were presented in the previous chapter and although correlation of the 2 tests was not 100%, the proportions seroconverting were comparable.

The month-county unit of sentinel chicken flock data was comprised of the number of individual birds which developed antibody for the first time divided by the number of susceptible (not previously infected) chickens within the county. The monthly bleeding schedules of chickens did not allow a clear-cut assignment of the month

a chicken converted from negative to positive antibody status (i.e., bleeding delayed until midmonth). However, an effort was made to have the apparent month of seroconversion correspond to actual month of initial antibody acquisition by the birds in a flock. Some counties had multiple flocks while many counties had none. Also the flock locations within counties did not remain constant for the entire 5 year period. The locations of rural sentinel flocks that were maintained during the present studies are presented in Table IV-1.

#### Temperature

Daily readings of the high and low recorded temperatures were available from Chico, Butte County; Marysville, Yuba County; Willows, Glenn County; and Bakersfield, Kern County. A monthly mean temperature (TMEAN) was calculated by summing the daily mean temperatures (mean of the daily high and low temperature) and dividing by the number of days recorded for the month. Only months having at least 15 days recorded were considered valid. Another measure of temperature (T0) was calculated by taking the monthly sum of the daily mean temperature from which a constant (5.3 C) was subtracted. A third measure of temperature (TMAX) was the monthly sum of the daily high temperature from which a constant (34 C) was subtracted. The constants were based upon experiments which determined the upper (34 C) and lower (5.3 C) limits of development of larval Cx. tarsalis in the laboratory (Reisen, Milby, and Bock, in preparation). Each of these measures was

taken in an attempt to determine the total amount of thermal energy accumulating in the environment (degree days) that might be related to the physiological requirements or constraints of Cx. tarsalis or the interaction of the vector and the virus. A similar approach has been used by others to study factors that affect the occurrence of epidemics of arthropod-borne viruses (Hess et al, 1963) and the activity of economically important pest species (Ring et al, 1983a).

Missing values were a problem for each of these temperature measures. This did not present an immediate problem for TMEAN but it can have a devastating effect on any attempt to use accumulating values such as T0 or TMAX. To avoid being overwhelmed with missing data, a compromise solution was used to normalize such months. This involved the use of the mean of TMAX multiplied by 30 for all months for which at least 15 observations were available. The use of a standardized 30 day month may have introduced a slight bias into the system but this bias was not particularly large and was preferable to the loss of much data. A further attempt was made to determine the effect of accumulated thermal energy by further summing the temperature measures T0 and TMAX for the entire year through the current month. These variables became TOTTO and TOTMAX, respectively.

#### Photoperiod

Photoperiod is an environmental variable which plays a major role

in the cyclic occurrence of some insect populations (Patton, 1963). An attempt was made to include photoperiod into the multivariate analysis of factors that affect viral occurrence. Two approaches were adopted. The first was a simple measure of the number of hours of light (HRSLITE) between the tabled time of sunup and sundown on the 15th of each month at 40 degrees north latitude (World Almanac, 1983). No adjustment was made for the latitude of the county in which the observations were made. The second approach was to determine the effect of the rate of change on the amount of light in the day. Two measures of rate of change of HRSLITE were used: the change in HRSLITE from midmonth to midmonth (CHMMO) and from the beginning to end of the month (CHBEN). Each was calculated by subtracting the number of hours of light in the appropriate day during the previous month from the number of hours of light at the appropriate point in the current month. Thus, when days were getting shorter, the values of these variables were negative and when days were getting longer they were positive. When the rate of change was great, such as occurred near the equinoxes, the magnitude of the variables were great while when the rate of change was low, such as near the solstices, the magnitude was small.

While photoperiod has a constantly repeated cycle from year to year it may have profound effects on the seasonal pattern of viral occurrence within a year. Effect on viral transmission may be related to behaviour of the vertebrate hosts, a variable not included in these studies. Photoperiod also may affect vector behavior through such pathways as length of the crepuscular periods during which Cx.

tarsalis feeds and the bifurcation of Cx. tarsalis populations into feeding and non-feeding subpopulations late in the summer season (Bellamy and Reeves, 1963; Reisen et al, 1983a)

#### Water resources

Rainfall. Total rainfall (mm) for the month (RAIN) was obtained by summing the daily rainfall for each of the sites where temperature data were recorded. The total rainfall (TOTRAIN) for the year was measured by accumulating the variable RAIN through the current month.

River flow. Water flowing through the Kern River channel at Stockdale Highway had been measured (acre-feet) at monthly intervals and was used as the variable RVRFLOW. Excess water results in flooding of water into sites that are favorable breeding sites for Cx. tarsalis.

#### Formation of a multivariate data set.

The individual data sets were formed and each time-space unit was identified as a month-county unit. The univariate data sets described above were merged by match-merging using the month-county units as the common identifying variable. The data included in the data set are summarized in Table IV-2.

### Analyses of data

Relationships between MIRs for the 3 viruses and the relative abundance of Cx. tarsalis were determined by product moment correlation (Box et al, 1978). A separate analysis was made for each county unit for the entire 60 month observation period. Realistically, isolation data were available for only a 4 or 5 month period each summer while LTI data generally were available from April through October. Correlations were performed of MIRs with LTIs from both urban and rural sites. Non-parametric correlations (Spearman-rank correlations) also were performed but the results were almost identical to the parametric Pearson correlations so only the Pearson coefficients are presented.

Similar product moment correlation coefficients were calculated to determine the relationship of chicken seroconversions for TUR and WEE viruses and the MIRs for these viruses from Cx. tarsalis within the month-county periods.

For counties where the AVRU previously had accumulated meteorologic data, a linear multiple regression analysis was done which included the LTI of Cx. tarsalis, meteorologic variables, and photoperiod. Variables were included in a stepwise procedure directed to choose the best 5 independent variable model which maximized  $R^2$  (SAS Institute, 1982). This was done for 2 reasons: to compare the relative predictive value of a set of variables for each of the 3



viruses being studied, and to determine the predictive value of multiple linear regression models that utilized the available data.

These analyses were performed on data available from 2 geographical areas: Kern County data only and combined data from the Glenn, Butte and Sutter-Yuba counties that were adjacent to each other. One problem in these analyses was the absence of WEE virus from the northern combined county unit during some time periods. This may have reduced the predictive value of the variables included in the model not because they do not affect WEE viral transmission or maintenance but because they had no opportunity to do so in the absence of the virus.

Field evaluation of transovarial transmission  
of HP, TUR, and WEE viruses.

In addition to the previously described laboratory studies (Chapter II), an attempt was made to gather evidence of transovarial transmission of viruses in Cx. tarsalis during 1983 in Kern County. Male mosquitoes were collected at sites where female mosquitoes were being collected for viral isolations. The males were collected from light traps, by aspiration from artificial and natural resting locations, and by sweep netting from swarms which form at sunrise and sunset (Reisen et al, 1983). The males were pooled and tested for virus in the same manner as female mosquitoes at the VRDL. For probability calculations, the occurrence of each virus was assumed to

be independent from the others.

## RESULTS

### Description of viral isolations

A total of 501,351 *Cx. tarsalis* were tested for virus. The statewide MIRs for each of the 5 years are given in Table IV-3. The rates for the entire 5 year period were highest for WEE (1.02) with HP and TUR being approximately equal 0.62 and 0.63, respectively. The MIRs for HP and TUR seemed to have a generally increasing trend over the 5 year period while WEE MIRs fluctuated. The high and low isolation rates of the 3 viruses did not correspond to each other as WEE and TUR had their highest isolation rates in 1982 while the high for HP was in 1981. The lowest isolation rate for HP was in 1979 while the lowest for WEE and TUR occurred in 1980.

Monthly MIRs for the entire state (Table IV-4) indicated that HP viral infections peaked in June and July while TUR virus is more evenly distributed from May through September. WEE virus has 2 peaks, 1 early in the year in June and another later in the summer during August and September. This may be an artifact created by separate peak WEE MIRs in the southernmost and Central Valley counties. Likewise, the combination of TUR data from the southern and northern portions of the State may create the impression that transmission is more evenly

distributed throughout the summer than actually occurs within the confines of a single county.

Yearly and monthly seroconversion for sentinel chickens for TUR and WEE viruses (Tables 3 and 4) roughly paralleled their respective MIRs. However, the ranks of the MIRs and conversions in chickens did not correlate directly. TUR viral antibody conversions in chickens had a consistent lag behind peak MIRs in Cx. tarsalis. This did not seem to be the case for WEE virus.

The distribution of MIRs over the counties in which samples were tested is presented in Table IV-5. The collections of Cx. tarsalis for viral tests were not evenly distributed throughout the State. In fact, 358,724 or 71.6% of the Cx. tarsalis were collected in 6 counties: Butte, Imperial, Kern, Riverside, Tulare, and Sutter-Yuba. If 2 more counties, Sacramento-Yolo and San Bernardino, are added to these 6 they account for 81.7% of the total Cx. tarsalis collected for viral tests during this period. Further description will be simplified and reliability increased by limiting analyses to the 6 counties that represented 71.6% of the collections.

HP MIRs were much lower in Imperial and Riverside counties in the south (0.0 and 0.12) than in Kern and Tulare counties in the San Joaquin Valley (1.34 and 1.59) and Butte and Sutter-Yuba in the Sacramento Valley had intermediate MIRs (0.24 and 0.18).

The distribution of TUR viral MIRs differed from those of HP. The highest MIRs were in Kern (0.93) followed by Sutter-Yuba (0.68), Tulare (0.67), Imperial (0.46), Riverside (0.42) and finally Butte (0.19). San Bernardino county had the highest isolation rate for TUR

virus (1.40) of any of the counties from which a sizable number of Cx. tarsalis were tested.

WEE MIRs were highest in the 4 southernmost counties with Imperial (2.34) having the highest followed by Tulare (2.28), Riverside (1.28), and Kern (1.23). The 2 Sacramento Valley counties were much lower, Butte with 0.33 and Sutter-Yuba with 0.25.

The yearly isolation rates from these 6 counties are listed in Table IV-6. HP MIRs were higher in the latter than in the earlier years of the study. Three of the counties had their highest MIRs in 1982 while 2 were highest in 1981. The remaining county, Imperial, had no HP viral isolations during the period of observation. There were no apparent consistent trends in the rates of WEE and TUR viral isolation.

The rural LTIs for these 6 counties also are presented in Table IV-6. The Sacramento Valley counties consistently had higher seasonal LTIs than San Joaquin or the southern counties. This did not result in the 3 viral MIRs being higher in the northern counties.

Detailed monthly MIRs for the 6 counties are presented in Tables IV-7 to IV-12. These tables describe both the monthly MIRs and the seroconversion rates of chickens. Examination of these tables emphasize that the numbers of mosquitoes tested for virus in any month was not large even for these 6 counties which represented 71% of all the Cx. tarsalis tested.

Sentinel chicken seroconversions.

Annual transmission rates of TUR virus to sentinel chickens has remained fairly constant statewide, ranging from 3.57% to 7.73% (Table IV-3.). WEE viral seroconversions were less steady as the highest seroconversions occurred in 1979 (6.18%) while the lowest occurred in 1981 (1.67%). Transmission of TUR virus to sentinel chickens each month had a definite late summer peak in August and September (Table IV-4.) Perusal of Tables IV-6 to IV-12 seem to confirm these patterns in the 6 counties from which the majority of Cx. tarsalis were collected.

#### Correlation of LTIs with MIRs and chicken seroconversions

Correlation was attempted of monthly MIRs of HP, TUR and WEE viruses and the monthly rural LTIs. The mean values for each variable and the correlations are summarized in Table IV-13. Many of the counties had few month-county units which contained corresponding MIRs and rural LTIs. This usually reflected the lack of testing for virus. In the 6 counties identified for more extensive analyses there was significant ( $p < 0.05$ ) correlation between TUR MIRs and rural LTIs in 2, Riverside and Sutter-Yuba counties. However, a large number of bivariate correlation procedures were performed and one would expect to find significant correlation coefficients occasionally at the  $p = 0.05$  level. No correlations were found for HP or WEE MIRs and the

LTIs for the month-county units. Numbers were small in all but the 6 counties with most numerous collections of Cx. tarsalis so correlation values ( $r$ ) had to be high to indicate a significant finding. Even though numbers were small, there were many negative correlations between mosquito abundance and MIRS. This seemed to be more common for correlations between TUR and HP MIRS and LTIs than for WEE. There were 4 negative correlations for WEE, 8 for TUR, and 8 for HP.

When correlations were attempted for the whole state, numbers for comparison of MIRs and rural LTIs became large. However, there still was no significant correlation between Cx. tarsalis abundance and the isolation rates for TUR or WEE virus. HP viral MIRs and rural LTIs were significantly correlated but in a negative direction.

Evaluations of urban LTIs and the MIRs of the 3 viruses did not increase the number of counties with statistically significant correlations. This indicates that at the county level the urban LTIs were no better for correlation with viral activity than the rural indices of vector abundance.

Correlation was attempted between monthly MIRs with transmission of TUR and WEE viruses to chickens (Table IV-14). Four of the 6 counties selected for detailed study had significant correlation between the MIRs and the seroconversion rates of the chickens, Butte, Kern, Tulare, and Sutter-Yuba. None of these 6 counties had significant correlation between TUR MIRs and chicken seroconversions. This may be due partially to a time lag in the seroconversion of chickens behind the infection rates. This lag is apparent by inspecting the seroconversion rates of chickens and the MIRS for TUR

virus in the more detailed Tables IV-6 through IV-11, particularly for Kern County. This relationship may be responsible for TUR viral seroconversions having negative correlations with MIRs in 8 counties in which chicken flocks had been maintained at 1 time or another. The same 8 counties had positive correlations for WEE virus in the corresponding periods. Two counties, Placer and Tehama, had positive significant correlation between TUR viral MIRs and sentinel chicken seroconversions for TUR virus. Statewide correlations of these 2 variables were positive and significant for both TUR and WEE virus. The numbers of variable pairings for the whole State were considerably larger than for the county comparisons and resulted in statistical significance in spite of relatively low correlation values (0.18 for TUR virus and 0.33 for WEE virus).

Analysis of the effect of environmental  
and vector abundance on viral MIRs.

Twelve variables that have been hypothesized to have an effect on viral infection rates were entered into a multiple linear regression model. Analyses for the 2 geographical areas constituted by 3 Sacramento Valley counties and Kern County for HP MIRS are presented in Table IV-15. The overall explanation of variation of HP MIRs for the 5 year period was significant for both areas. However, selection of independent variables which best explained variation was somewhat confusing. LTI (urban) was selected as a predictor variable but in 1

area its partial correlation coefficient was positive while in the other it was negative. This probably indicates a geographical dissimilarity of monitoring sites between areas for viral isolation and population monitoring. TOTRAIN was selected as a predictor variable in the north while it was not selected for Kern County. Both TOTTO and TOTMAX were selected in both areas and the direction and magnitude of predictive value was similar. One variable describing photoperiod was chosen in each area of analysis. CHMMO was chosen in the north while HRSLITE was selected in Kern County.

Analysis of TUR MIRs with the same group of predictor variables was less efficient in generating a predictive model (Table IV-16). Rural LTIs were chosen in both locations and again the partial correlation coefficient was positive for the Sacramento Valley location but negative for Kern County. TMAX was chosen in both locations but again its influence was positive in 1 location and negative in another. TOTRAIN was chosen in both locations and had a negative effect on TUR MIRs. RAIN was chosen in Kern County and had a positive effect on TUR MIRs. HRSLITE and TOTMAX were selected into the model in the north and not in Kern County. The overall variation explained was approximately the same in both locations. However, the number of month-county units examined was small and the significance of the models' explanation of the overall variation was no greater than expected by chance alone ( $p > 0.05$ ).

Similar analyses of the predictive potential of the variables for WEE MIRs (Table IV-17) indicated that the 5 variable model predicted more variation than expected by chance alone for Kern County but not



for the northern counties. This probably reflects the reduced or total lack of WEE viral activity in the northern area during the latter part of the 5 year observation period and this did not allow the variables to affect WEE MIRs simply because the virus was not present and free to be influenced by these factors. In the northern area the variables selected into the model were both the rural and urban LTIs. Rural LTIs had a negative partial correlation coefficient while the urban LTIs had a positive effect. Other variables selected were RAIN, TOTMAX and TOTTO. The standard errors of the partial correlation coefficients were large in all but urban LTIs. Therefore, the statistical significance of the overall model was low ( $p=0.29$ ) even though the  $R^2$  was relatively large (0.30). The predictor variables selected into the Kern County model did not include the LTIs. Rather, RAIN, TMAX, and all 3 of the photoperiod variables, HRSLITE, CHMMO, and CHBEN were selected into the model. The effect of high temperatures (TMAX) was negative as was CHMMO. The remaining 3 predictor variables all positively influenced WEE MIRs. The overall amount of variation described for WEE viral MIRS was large ( $R^2=0.74$ ) and the statistical significance of the model was greater than expected by chance ( $p=0.0001$ ). The comparative increase in model efficiency from the northern area to Kern County probably reflected that WEE virus was present in Kern County every year.

One additional variable, RVRFLOW, was introduced into the multivariate linear model for Kern County. However, RVRFLOW was not selected into a model that included the other predictor variables, indicating that it had little predictive value for the monthly MIRs of

any of the 3 viruses in Kern County.

Field evaluation of transovarial transmission of TUR  
and HP viruses in Kern County

A large number of male and female mosquitoes were collected simultaneously from 4 sites in Kern County (Breckenridge, Kern River, Poso West, and John Dale Ranch study areas). A total of 113 isolations of HP virus and 25 of TUR virus (Table IV-18) were made from these collections. There was 1 isolate of HP virus from a pool of male Cx. tarsalis at the Breckenridge site. The rarity of HP viral isolations from male Cx. tarsalis indicated either that this was an invalid isolation or that this virus is not transmitted transovarially with the same efficiency to male and female progeny. No TUR or WEE viral isolates were made from male Cx. tarsalis. The numbers of males and females collected were high enough to strongly infer that transovarial infection is unusual for these 3 viruses.

#### DISCUSSION

The trends of viral occurrences of the 3 viruses allow some inferences to be made about their maintenance mechanisms. WEE virus occurred regularly in the southern counties of California. However, even in these counties the pattern of monthly WEE MIRs was erratic and

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TURLOCK VIRUSES IN CALIFORNIA(U) AIR FORCE INST OF TECH  
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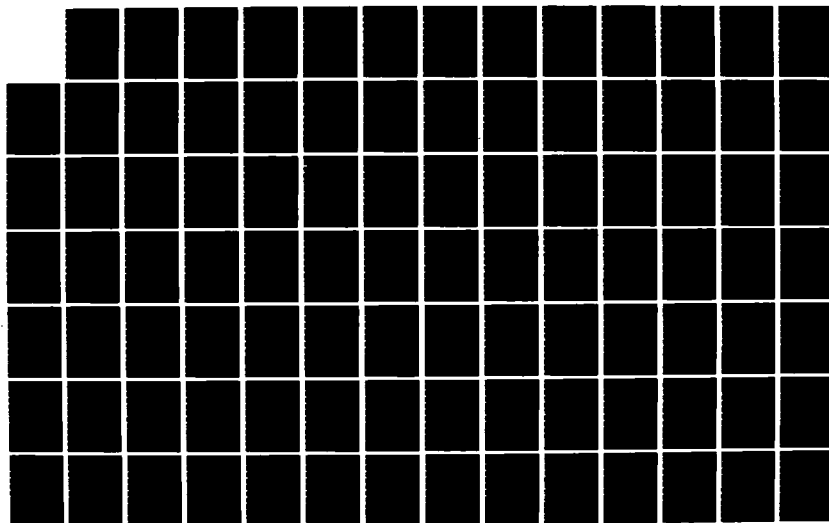
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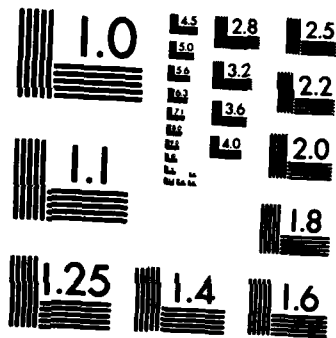
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varies widely annually. On the other hand, both TUR and HP viruses were more consistent in their occurrence in most locations each year. Furthermore, TUR virus was more evenly distributed throughout the entire summer than HP or WEE virus. This could indicate the virus is transovarially transmitted and is maintained at a steady level in the vector population (Fine, 1975) or that the virus is continually seeded into the vector population by another mechanism such as chronically infected vertebrate hosts. On the other hand, HP virus peaked during June and July. This peak may indicate that amplification of the virus occurs and that transovarial transmission is not the only means for viral transmission from generation to generation of the vector. The pattern of WEE viral occurrence was more varied than the other viruses. It generally did not appear until later than HP or TUR viruses and once it appeared the MIRs increased much more rapidly than for the other 2 viruses. The WEE pattern is in keeping with traditional views of the amplification of an arbovirus through vertebrate hosts.

The occurrence of a period of high MIRs during the course of the summer does not preclude TOT of virus in the vector but it does indicate that it is not the only mechanism that allows passage of virus between vector generations. Until recently, it was thought that those viruses which undergo TOT of virus still relied upon some viral amplification in vertebrate hosts each summer as TOT rates in the population were too low to maintain a virus indefinitely within the vector population (Fine, 1975; Fine and LeDuc, 1978). It is now known that there are mosquito "clones" that are nearly 100% efficient in

transmitting virus to their progeny (Tesh, 1980; Turell, 1982b). However, the field investigation of infection in male Cx. tarsalis indicates that TOT is not occurring or is very rare for WEE, TUR, or HP viruses. In those viruses for which TOT has been demonstrated, the infection rate of male and female progeny has been shown to be approximately equal (Watts et al, 1973; Tesh and Chaniotis, 1975; Rosen et al, 1978; Hardy et al, 1980; Turell et al, 1982). Therefore, it would be expected that the isolation rate of TUR or HP virus would be similar from both sexes unless infection has an extreme effect upon survival or behaviour of the males. Isolation rates from males and females were significantly different in the present studies.

Attempts to correlate the abundance of Cx. tarsalis with the MIRs of each of the 3 viruses were not very successful. This probably was the result of attempting to use data collected from a surveillance program that was designed to determine merely if virus was absent or present at low, medium, or high levels. The LTIs which were used as a measure of the relative abundance of Cx. tarsalis may not have been sensitive enough to detect critical fluctuations in population levels. The averaging of a number of LTIs from trapping locations spread over large geographical areas such as counties probably diminished the value of this index for any specific location. In addition, the collection of mosquitoes for viral isolations could only be done at sites where sufficient mosquitoes were available to result in pools for testing. This undoubtedly led to a bias towards sampling from rural sites where mosquito numbers were high while LTIs frequently were derived for distant sites that did not experience the

same population extremes. This same problem probably existed in attempts to correlate LTIs with serological conversions in chicken flocks. Maintaining, bleeding and testing the chickens is expensive so the number of flocks in any given county was very limited (with the exception of Kern County). Some flocks were located in areas to detect viral activity where collections of mosquitoes were small and it was impractical to accumulate pools to determine MIRs. So the numbers of mosquitoes present at the site of some sentinel chicken flocks was low while the numbers probably were high at locations where mosquitoes were collected for viral tests but there were no sentinel flocks. These disparities probably explain the low degree of correlation between MIRs for TUR and WEE viruses and seroconversions in sentinel chickens.

It probably is unreasonable to expect to find significant correlations between MIRs and LTIs or chicken seroconversions. Previous studies indicated the minimum levels of Cx. tarsalis that were required to maintain and amplify WEE and SLE viruses (Reeves, 1967; Reeves, 1970a). These studies were done in circumstances where measures of the above variables were made concurrently at the same site and in a limited geographical area. This type of study has much to be said for it because the relationships that can be discerned are less subject to the type of sampling errors that widespread sampling of a less intensive nature create. On the other hand, such focused studies may lack generalizability to a broader area of concern. The present studies attempted to apply the same principles to data derived from a completely different set of circumstances.

Another apparent problem is that it is very difficult to determine the effect of variables on the levels of virus in a vector population when the virus is not present in the population for at least a part of the period. This was particularly true in the northern counties for WEE virus. This virus was present at very low levels or not at all during 1981 and 1982. The lack of virus may or may not have been related to the levels of the variables even during the time of the analysis. If virus is not present in an area and must be introduced, the local variables are not able to have as much effect as they would if virus were continually present. None of the independent variables used in the multivariate model could be expected independently to explain all of the variation in MIRs in any of the viruses. This is a primary reason for using a multivariate model, to investigate the effect which a combination of conditions has upon viral activity.

Conclusions on the dependence of the 3 viruses upon levels of Cx. tarsalis for maintenance of the virus are precluded by the lack of correlations between Cx. tarsalis levels and MIRs. The disappearance of WEE and SLE viruses from most of the northern counties in recent years while HP and TUR viruses persisted indicate that the long term maintenance mechanisms for TUR and HP viruses are more efficient than those for WEE and SLE viruses. Monthly patterns of isolation still indicate that HP virus is being amplified by a vertebrate host. The pattern of amplification, with peak isolation rates in June and July, may indicate dependency for amplification upon a vertebrate host which is present in peak numbers at a corresponding time. This might not



only reflect both the presence of the species and the presence of a certain age-class of the host. This would be expected if there is an age restriction of viral replication to very young animals, such as nestling birds, or if the number susceptible animals which remain in the population diminishes as infected individuals become immune. Flanders virus was not isolated from birds in the Mid-West until the isolation program shifted emphasis from adult birds to nestlings (Kokernot et al, 1969). In an area of highly endemic viral activity this could reflect either high antibody prevalence in adult birds or restriction to nestling birds of efficient viral replication.

House finches had a high prevalence of HP antibodies in Kern County (Chapter III). This species was the second most numerous avian species in Kern County in the late 40s and early 50s (Reeves and Hammon, 1962) and the house finch was the source of blood meals for over 30% of the approximately 4,000 Cx. tarsalis which had taken passerine blood meals and for which blood meal identification was taken to species level (unpublished).

TUR MIRs in Cx. tarsalis were more evenly distributed than were WEE and HP MIRs and this may be because an essential vertebrate host was present for the entire summer or developed an infective viremia either as adults or nestlings. It is also possible that chronically infected birds were capable of infecting Cx. tarsalis.

As mentioned in the introduction, a large number of variables that can influence the amplification and maintenance of an arthropod-borne virus have been identified (Reeves, 1967). It is clear that the present analyses did not include many factors which can have

a major and direct impact on MIRs in the vector population. One of the most important of these is the number of susceptible vertebrate hosts that are present in an area. If TOT does not occur, as the isolation attempts from males and laboratory data (Chapter II) indicate, then the availability of such hosts is of paramount importance. It may be that the availability of vertebrate hosts is a limiting variable that has greater influence than the number of vectors.

The 1 isolation of HP virus from a pool of males may not be valid. The isolation rate from males was far below that expected if TOT is equally efficient in male and female *Cx. tarsalis*. Virus was reisolated from the original pool but it is always possible that the pool was mislabelled or that 1 or more females or parts of females were included in the pool. The lack of transovarial transmission in laboratory attempts and the low frequency of isolation of HP virus from males in an area where the isolation rate was high among females both support the contention that the single isolation from a pool of males was something out of the ordinary.

The use of multivariate linear models to evaluate the predictive value of vector and environmental variables met with mixed success. On one hand, the models explained a large portion of the overall variation of the MIRs for all 3 viruses in both the northern areas and Kern County. On the other hand, the use of monthly periods of observation and limitation of the period of observation to 5 years gave a relatively small number of month-county periods for inclusion in the model. This resulted in a borderline significance for 2 of the

6 ( $.05 < p < .20$ ) models and less precision ( $p = .29$ ) in 1 other evaluation. The use of a computer program which selected the "best" 5 independent variables for prediction was an expedient way to select those variables which had the most influence upon the viral MIRs. One would hope that these predictor variables would have the same influence from area to area and within an area over time. However, the period of observation was not long enough to rigorously evaluate any model developed by the use of derived parameter values in an independent sample of data. As mentioned above, the period was barely long enough to lend significance to the predictive qualities of the present model.

Many of the environmental variables used in these models may not have a direct effect upon either the virus or the mosquito vector. Rainfall, temperature, and photoperiod all may have an effect on vertebrate hosts that are as important as vectors for amplification and maintenance of the viruses.

Another criticism of this type of analysis over a limited period of time is that it may not be a period that includes extreme viral activity. Previous investigations in which variables were associated with viral trends usually encompassed a much longer time period including epidemic years when there were high rates of viral activity (Reeves and Hammon, 1962; Hess et al, 1963; Olson et al, 1979). Such approaches allow much greater opportunity for variables to co-vary.

## V. OVERALL DISCUSSION AND CONCLUSIONS

Serologic findings and evaluation of the IFA test

HP and TUR viruses occur with a high frequency each year in Cx. tarsalis in California (Emmons series). Thus it would appear there is a potential for vertebrate populations to be exposed to these viruses. During the last 5 years the viruses have occurred in Cx. tarsalis in a much more regular fashion than either WEE or SLE viruses. These observations have posed interesting problems that the present studies have attempted to answer. The first problem was to determine the prevalence of infection with TUR and HP viruses in various vertebrate species. An associated problem was to determine if these viruses were causing CNS disease in man or horse. A third problem was to explain the annual persistence of these viruses.

Current and earlier serological tests determined the extent of infection in a range of vertebrate hosts. Infection of man with TUR virus is a rare event. Only one of approximately 2000 humans had IFA antibody to TUR virus. Previous results (unpublished) with the HI test and confirmatory PRN tests with a smaller sample of 236 sera supported the IFA findings. There were no HP or TUR IFA positive sera in a cross-sectional sample of humans taken in Kern County in 1960 and this population had relatively high antibody prevalences for WEE and SLE viruses (Froeschle and Reeves, 1965). This same population had

frequent exposures to the bite of Cx. tarsalis (Reeves and Renteln, 1959). HP IFA antibody was not found in any of the human sera tested. However, in the 236 CNS cases tested for PRN antibody, 13 individuals had HP PRN reactions in their sera and 5 of these 13 had 4-fold or greater titer rises. The patterns and titers of these reactions and lack of confirmatory IFA antibody make the neutralizing substance of questionable specificity. One can infer from these serological findings that HP and TUR viruses almost certainly are not important causes of infection or morbidity in man in California.

TUR viral infection of horses is a fairly common event as 25% of 499 horses tested from a cross-sectional sample collected in the Central Valley in 1968 had TUR IFA antibody. Other studies using the PRN test (unpublished) and limited comparisons of the IFA with the PRN test within these studies indicated that the true prevalence of past infection of horses with TUR virus was even higher than 25%. However, no rise in titers to TUR virus was found in sera from 146 equine CNS cases. Only 2 of 499 horses had serological evidence of past infection with HP virus by IFA test. Limited PRN tests on a subsample of these animals confirmed that relatively few infections had occurred. No rising titers to HP virus were found in sera from 146 equine CNS cases from which sera were examined.

Serological evidence of infection with HP and TUR virus was found in sera from dogs sampled in 1968 and 1983. HP antibody prevalence was higher 35/44 (80%) in 1968 than in 1983, 10/77 (13%). The TUR antibody prevalence also was higher in 1968, 13/44 (30%), than in 1983, 11/75 (15%) However, the animals for each of the time periods were from

different areas so direct comparisons have limited validity. It appears that infection with HP and TUR viruses was common in dogs. The nature of the samples did not allow interpretation to be made of the pathogenic potential of these viruses. However, infection was common enough to warrant future studies of the role of TUR and HP infections as possible etiological agents of illnesses in dogs.

There was little evidence of infection of cattle and sheep with HP or TUR virus. There was serological evidence of past infection of pigs with TUR virus as 5/61 (8%) were positive. The cross-sectional sample used did not allow any interpretation of the possible pathogenicity of these viruses.

The lifetimes of most domestic mammals are relatively long. If one accepts the premise that infection with viruses impart longterm immunity, the age structure of domestic animal populations limits their suitability to amplify either of these 2 viruses. When this characteristic is coupled with the low rates of infection, it must be concluded that these domestic species play little or no role in the maintenance of HP or TUR viruses in California.

Limited testing indicated that wild mammals rarely were infected with either virus. A similar finding was reported earlier for TUR virus in a study of jackrabbits in the Sacramento Valley (Hardy et al, 1977). Smaller rodents have a high reproductive rate which increases their potential to be effective hosts. However, Cx. tarsalis does not utilize small mammals as a blood meal source as commonly as it does birds or larger mammals (Reeves et al, 1963; Teampelis et al, 1965; Teampelis and Washino, 1967). It is concluded that rodents or

lagomorphs probably are not important maintenance or amplifying hosts for TUR or HP viruses.

Both HP and TUR PRN antibodies were found in the sera of house finches collected in Kern County between 1971 and 1973. This bird was found to be the second most numerous species in population surveys during the late 1940s and the early 1950s (Reeves and Hammon, 1962). This species is a preferred source of blood meals for Cx. tarsalis (unpublished), the reproductive season is long, and fledging success is relatively high. The population numbers, blood meal analyses, and reproductive success for the house sparrow are nearly identical to those of the house finch. However, no evidence of infection with either virus was found in the limited sample of house sparrows that were tested in these studies. More extensive earlier serological studies found TUR antibodies in the sera of house sparrows although not at the same high prevalence as in the house finch (unpublished). Moreover, in experimental infection studies, the house sparrow had lower TUR viremia levels than the house finch. It is concluded that the house finch is a more efficient vertebrate amplifying host for TUR virus than the house sparrow.

The potential for HP virus amplification in the house finch was not evaluated, but based on serological findings, studies should be done to determine the level and duration of viremia and the serological response in this species. Since transovarial transmission of HP virus could not be demonstrated in Cx. tarsalis, another area worthy of future investigation would be the potential that HP virus may develop persistent infections in the house finch.

The IFA test was reasonably sensitive for TUR antibodies when compared with the HI test but was not as sensitive as the PRN test. The IFA was less sensitive than the PRN test for the detection of HP antibodies. The IFA test may have lacked sensitivity because the hosts had been infected a year or more earlier and antibody titers had ebbed. The one exception was in the sentinel chickens as it was known that they had been infected with TUR virus, and possibly with HP virus, within a period of no more than 4 months before the sera were collected.

Mechanisms for viral persistence and evaluation of transovarial transmission

Mechanisms for TUR viral persistence have been studied previously including persistent vertebrate infection in the house finch model (unpublished) and TOT of the virus in vectors (unpublished). Virus was recovered from tissues of 2 house finches at 28 and 81 days post-infection using co-cultivation techniques at reduced culture temperatures (unpublished). However, Cx. tarsalis that fed on these persistently infected birds failed to become infected. Attempts to demonstrate TOT of TUR virus in Aedes dorsalis were unsuccessful (unpublished). This mosquito is capable of TOT of CE virus (Turell et al, 1982). Mechanisms for maintenance of HP virus had not been investigated previously. In addition other parameters of the ability of HP virus to replicate and be transmitted by Cx. tarsalis remained



unevaluated.

The present studies evaluated TOT of both HP and TUR viruses from experimentally infected female Cx. tarsalis to their larval and adult progeny. TOT was evaluated at 18 C and 27 C in Cx. tarsalis without finding the virus in progeny mosquitoes. These studies were designed to avoid the ability of normal larval tissues to decrease the capacity to detect viruses in mosquito pools (Ksiazek et al, 1983).

It is concluded that if the viruses are passing from 1 generation of Cx. tarsalis to the next, they are doing so at a very low rate. One might argue that the combination of viruses and Cx. tarsalis strains were not the proper combination for successful transmission of the virus. Evaluation of other Bunyaviruses with demonstrated TOT have shown that TOT rates may differ from virus to virus and mosquito population to mosquito population but the filial infection rates are generally high (Miller et al, 1982; Turell et al, 1982a). The possibility still remains that overall TOT rates of HP and TUR viruses are very low and that there are "clones" of highly efficient maternal transmitters within field populations as has been shown experimentally for San Angelo (Tesh, 1980) and California encephalitis viruses (Turell et al, 1982b). The filial infection rates for San Angelo and California encephalitis viruses have been relatively high in initial TOT attempts from experimentally infected female mosquitoes and this definitely was not the case for TUR or HP viruses.

It is possible, but unlikely, that other mosquito species that are not the "epizootic" amplifiers of TUR or HP virus may be responsible for the primary maintenance of viral infection and that

virus "spills over" into the Cx. tarsalis population after infection of a suitable vertebrate host fed upon by both mosquito hosts. There is little evidence to support this hypothesis because few isolates of either HP or TUR virus have been reported from species other than Cx. tarsalis over a period of many years (Emmons series; Berge, 1975).

TUR virus occurs at a relatively constant level in female Cx. tarsalis at most locations and there is a discernible summer increase in TUR MIRs. This pattern of TUR MIRs in the mosquito indicates that amplification of TUR virus occurs but that the infection rates do not "spike" in the same way as WEE and SLE viruses. The somewhat subdued seasonal peak in TUR viral MIRs is consistent with the combination of an efficient maintenance mechanism with a supplemental amplification cycle. Alternatively, the relationships of TUR virus, the house finch, and Cx. tarsalis may have co-evolved to the point that no annual "epornitic" of TUR viral infections occurs. No matter what the summer transmission cycle, TUR virus must still persist from one season to the next. Laboratory and field evaluation strongly reject the hypothesis that TOT is the maintenance mechanism responsible for TUR viral persistence. However, there is some evidence to support the hypothesis that the vertebrate host, in this case persistently infected house finches (unpublished), may offer an alternative mechanism for viral persistence as has been suggested by others (Reeves, 1974).

HP virus, although transmitted throughout the summer season, appears to have a peak of infection in female Cx. tarsalis during the months of June and July. In spite of extensive testing, only 1

isolation of HP virus was made from male Cx. tarsalis. The inequality of male and female HP viral isolations is further supported by similar findings for FLA virus in Connecticut (Main, 1981). These findings are consistent with the traditional amplification of a virus in a vertebrate-mosquito transmission cycle. However, this does not explain the maintenance mechanism for HP virus over the winter. If TOT is rejected as a primary mechanism for viral overwintering, it must be maintained by persistent infection in the vertebrate host or by reintroduction of virus from other areas. The regularity of HP viral activity each year in the same areas of California opposes the notion that the virus is reintroduced into the State annually from other sites.

The possibility that female mosquitoes take an infective blood meal and then overwinter is a remote possibility. Evidence indicates that almost all female Cx. tarsalis that survive the winter as inseminated females have not taken a previous blood meal and feed for the first time when diapause is broken in the early spring (Bellamy and Reeves, 1963; Reisen et al, 1983a). If TOT is not occurring and female Cx. tarsalis that feed on potentially infectious vertebrate hosts in the fall do not survive the winter, the virus must overwinter elsewhere than the mosquito.

Transmission of TUR and HP virus by the bite of Cx. tarsalis TUR and HP viruses both replicate when inoculated into Cx. tarsalis. TUR virus infects Cx. tarsalis following the ingestion of a viremic blood meal and transmits the virus to a susceptible vertebrate host following a suitable extrinsic incubation period (unpublished). HP

virus also can be transmitted by Cx. tarsalis that are infected by inoculation but this was only demonstrated by an artificial feeding technique. HP virus did not infect Cx. tarsalis when virus was ingested; however, the dose of virus that mosquitoes ingested was so low that one would not expect a very high infection rate unless Cx. tarsalis is very susceptible. The finding of a high prevalence of HP antibodies in the sera of house finches may indicate that this would be a suitable vertebrate host with which to attempt biological transmission cycles, as has been done for TUR virus (unpublished).

Dependence of viral maintenance and transmission upon Cx. tarsalis abundance

Month-county units of virus and vector occurrence were assembled to investigate differences in dependence of viral activity upon the relative abundance of Cx. tarsalis. These studies were prompted by previous studies which indicated there were threshold levels of Cx. tarsalis populations below which WEE and SLE viruses were not transmitted in the basic enzootic or in the epidemic/epizootic cycles (Reeves, 1967; Reeves, 1970a; Olson et al, 1979). The present studies found very poor correlations between the abundance of Cx. tarsalis and WEE, HP, or TUR viral MIRs. Transmission of TUR or WEE virus to sentinel chicken flocks also did not correlate well with MIRs of Cx. tarsalis. One reason for a lack of correlation between Cx. tarsalis LTIs and viral MIRs or viral transmission rates and MIRs probably was

the source of the data. The month-county figures represented a compilation of weekly data from many locations in MADs within the counties. Averaging these figures into a summary abundance figure undoubtedly resulted in loss of individual collection site trends. However, the numbers of mosquitoes collected for viral tests did not allow the use of smaller time units. Data coding did not allow the use of smaller geographical units. On the positive side, one could presume that higher and broadly distributed mosquito populations are required for virus to cause major epidemics or epizootics.

Another circumstance that probably limited the capacity to correlate mosquito abundance with viral infection rates was the short period of observation, 5 years. During this period WEE virus remained at a relatively low level. HP and TUR viruses seemed to increase slightly in prevalence in the Cx. tarsalis population during this time. Exceptional environmental conditions and either higher seasonal abundance or higher subseasonal peaks in Cx. tarsalis populations may be necessary to significantly influence viral transmission rates. The period under observation may have lacked the extremes in populations necessary to influence viral infection rates.

Multivariate approach to prediction of viral activity The same variables used in the univariate correlations in the month-county units were expanded into a multivariate model by adding environmental measurements such as temperature, rainfall, and photoperiod. Analyses were confined to a contiguous group of Sacramento Valley counties and Kern County. The addition of the other variables to the univariate model greatly increased the possibility of detecting factors that

might influence variations in the viral infection rates in the Cx. tarsalis population. Cx. tarsalis LTIs did not enter into the model consistently when a method was applied to maximize the amount of variation explained by a particular model. Instead, measures of temperature or photoperiod were more consistently selected as independent variables in the model. No clear picture emerged of the dependence of the 3 viruses on a common independent variable. The same criticisms that applied to the univariate correlation analyses also apply to these attempted multivariate analyses: the period of observation is very short (5 years), the years seem to have been average inter-epidemic years for WEE virus transmission, and WEE virus was not present in the Sacramento counties for all of the observation period.

Probably the most serious handicap in the multivariate analyses was a lack of information about other variables that are critical to transmission of the virus through a mosquito-vertebrate cycle. The house finch is known to be a suitable vertebrate host for WEE and TUR viruses and it is suspected that a similar situation may exist for HP virus. The abundance and immune status of the vertebrate component of the viral transmission cycle may represent more limiting factors to the cycle than vector abundance. However, no data were available on the vertebrate host variables. Other critical factors which can have a profound effect on the ability of a virus to be transmitted in a given area are other mosquito parameters not available during these studies. For example, vector competence has been shown to vary in populations of mosquitoes (Hardy et al, 1983) and susceptibility to

oral infection of Cx. tarsalis with TUR virus has been shown to differ between geographical populations (unpublished). Such susceptibility has been shown to be genetically controlled for WEE virus and susceptibility to oral infection with WEE virus is genetically independent of oral susceptibility with TUR virus (Hardy et al, 1978). Whether there are genetic differences in the susceptibility of Cx. tarsalis to TUR and HP viruses has not been investigated. Another variable which may greatly influence the vectorial capacity of Cx. tarsalis is the autogeny rate in a population. The delay in first blood meal that is associated with autogeny drastically reduces the probability of transmission of a virus, unless TOT occurs. This is because autogeny delays the ingestion of an infectious blood meal and viral transmission. Analyses of female Cx. tarsalis lifetables and application of survival probability (and transmission probability) to the viral cycle have clearly demonstrated this concept (Reisen et al, 1983a).

Representation of possible cycles of transmission and maintenance of HP and TUR virus.

Figurative diagrams of the possible transmission and maintenance pathways for TUR and HP viruses have been generated to assist in visualization of areas of possible future research (Figures 1 and 2). The cycles are tentative and sketchy but make use of the available information presented in the above discussions.

#### Areas for future investigation

A primary objective of future studies should be to investigate the potential of vertebrate hosts to act as amplifiers of HP virus. Cx. tarsalis host feeding preference, and the serological evidence of HP infection in the house finch would indicate that birds should be the primary focus of such studies. House finches and house sparrows would be the first species to evaluate for their viremia patterns, ability to infect Cx. tarsalis while viremic, and documentation of their serological response to infection.

Non-traditional reservoirs of viral persistence should also be kept in mind in future studies if none of the more common mechanisms occur at high rates for HP or TUR virus. Possible mechanisms for consideration are infection of other hematophagous invertebrates, internal parasites, or even infection of plants or aquatic organisms upon which mosquitoes feed at various stages of their lifecycle. Many of these mechanisms have been speculated on as reservoirs for VSV viruses which appear to be maintained in a basic TOT cycle in sandflies in tropical regions (Tesh, 1975). TUR and HP viruses may persist in Cx. tarsalis in forms which are not easily isolated by the techniques currently employed and only after the virus begins the summer amplification cycle through vertebrate hosts are viral variants accessible. Support for this hypothesis was found when WEE viral isolates with low pathogenicity for hosts employed normally for viral



isolation were obtained from Cx. tarsalis collected during the winter months in Kern County (Reeves et al, 1958).

A test more sensitive than the IFA or HI for detection of HP and TUR antibodies would be desirable. The micro-spot technique of the IFA test used in these studies allowed the screening of sera against multiple viral antigens. The EIA offers greater sensitivity for detection of antibody to a great variety of infectious agents (Yolken, 1980) and could greatly facilitate further HP and TUR viral studies. Recently, a micro-dot EIA performed on nitrocellulose membranes has been described (Pappas et al, 1983) and this technique would lend itself to screening of multiple antigens in a single test. The problem remains that each of these tests rely upon indirect techniques that depend on use of a second anti-species antibody to screen sera from multiple species. This is a particularly acute problem in studies involving arboviruses or other zoonotic agents where numerous wildlife species can be involved.

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TABLES

Table II-1. Histories of Hart Park and Turlock virus strains used in experimental studies.

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Virus  Strain          Source              Passage history [a]
-----
TUR    (847-32)           Cx. tarsalis      E1,SMB10
TUR    (FMS4783)          Cx. tarsalis      SMB3
TUR    (Kern 82-63)       Cx. tarsalis      Cx. tarsalis i.t. 1 or 2
HP     (AR70)              Cx. tarsalis      SMB19
HP     (BFN5662)          Cx. tarsalis      V2,SMB2,V6
HP     (Kern 83-5350)     Cx. tarsalis      Cx. tarsalis i.t. 1 or 2
=====

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[a] SMB=suckling mouse brain, E=chicken embryo, V=Vero, i.t.=intrathoracic inoculation.

Table II-2. Comparison of the sensitivity of different cell lines for the detection of Hart Park (BFN5662) and Turlock (847-32) viruses.

| Cell line | Origin              | Passage level | Titer (log pfu/0.1ml) |                  |
|-----------|---------------------|---------------|-----------------------|------------------|
|           |                     |               | HP (BFN5662) [a]      | TUR (847-32) [b] |
| DECC      | Pekin duck          | Primary       | <1.0                  | 6.5              |
| Vero      | Vervet kidney       | 153           | 4.3                   | 5.0              |
| BSC-1     | Vervet kidney       | 49            | <1.0                  | 4.9              |
| CER       | Hamster kidney      | 91            | <1.0                  | 4.4              |
| BHK(0853) | Hamster kidney      | 327           | [c]                   | [c]              |
| LLC-MK2   | Rhesus kidney       | 115           | <1.0                  | 4.5              |
| SIRC      | Rabbit cornea       | 437           | <1.0                  | [c]              |
| Bat lung  | Free-tailed bat     | 70            | <1.0                  | 5.0              |
| PS        | Pig kidney          | 112           | <1.0                  | 5.5              |
| XTC-2 [d] | African clawed toad | 530           | <1.0                  | 3.80             |
| MDCK      | Dog kidney          | 58            | <1.0                  | <1.0             |

[a] Virus stock was Vero2, SMB2, Vero6.

[b] Virus stock was Egg 1, SMB10.

[c] Plaques evident upon microscopic examination but not distinct enough to enumerate. BHK (0853) cells did not take up neutral red.

[d] Assayed at 28 C.

Table II-3. Comparison of the sensitivity of 2 cell lines, Vero and C6/36, and intrathoracic inoculation of Cx. tarsalis for detection of Hart Park (BFN5662) virus.

| =====                |                 |                         |
|----------------------|-----------------|-------------------------|
| Titer log per 0.1 ml |                 |                         |
| 10                   |                 |                         |
| -----                |                 |                         |
| Vero cells (pfu)     | C6/36 cells [a] | <u>Cx. tarsalis</u> [b] |
| -----                |                 |                         |
| 3.12                 | 3.25            | 4.85                    |
| =====                |                 |                         |

[a] Infection status of C6/36 cells determined by direct fluorescent antibody test with anti-HP (AR70) conjugated antiserum. Titer (TCID ) 50 determined by the method of Reed and Meunch (1938).

[b] Individual mosquitoes assayed for presence of virus by plaque assay on Vero cells. Titer(ID ) determined by method of Reed and Meunch 50 (1938) upon groups of 10 mosquitoes/dilution.

Table II-4. Oral transmission of Hart Park (BFN5662) virus to droplets by *Cx. tarsalis* following intrathoracic inoculation and extrinsic incubation at 27 C. [a]

| Experiment | Days<br>post<br>inoculation | Transmission to droplets |              |       |
|------------|-----------------------------|--------------------------|--------------|-------|
|            |                             | Positive (%)             | Negative (%) | Total |
| I [b]      | 14                          | 1 (6)                    | 17 (94)      | 18    |
|            | 21                          | 10 (34)                  | 19 (66)      | 29    |
|            | Total                       | 11 (23)                  | 36 (77)      | 47    |
| II [c]     | 14                          | 8 (42)                   | 11 (58)      | 19    |

[a] Includes only mosquitoes that had fed to 1/2 or more of repletion.

[b] Assayed by plaquing on Vero cells.

[c] Assayed by plating of droplets on C6/36 cells and assay by direct fluorescent antibody test at 1 week post infection.

Table II-5. Attempt to demonstrate transovarial transmission Turlock (FMS4783) virus in Cx. tarsalis (Knights Landing Colony) following intrathoracic inoculation. [a]

| Ovarian cycle | Stage      | Virus isolations/<br>No. pools tested | No. individuals tested |
|---------------|------------|---------------------------------------|------------------------|
| 1             | 4th instar | 0/178                                 | 4,398                  |
|               | Pupae      | 0/32                                  | 671                    |
| 2             | 4th instar | 0/14                                  | 343                    |
|               | Pupae      | 0/3                                   | 49                     |
| Total         | 4th instar | 0/192                                 | 4,741 [b]              |
|               | Pupae      | 0/35                                  | 720 [c]                |

[a] Female mosquitoes inoculated with virus, incubated at 18 C for 7 days, fed on chicks, fed mosquitoes subsequently held for 7 days at 18 C, and then allowed to oviposit. Egg rafts pooled, hatched, and larvae reared to 4th instar or pupae at 18 C.

[b] Can reject the null hypothesis that the true "infected" population proportion, p=0.0006 at alpha=0.05 level.

[c] Can reject the null hypothesis that the true "infected" population proportion, p=0.005 at alpha=0.05 level.

Table II-6. Attempt to demonstrate transovarial transmission of Hart Park (BFN5662) virus in Cx. tarsalis (Knights Landing Colony) after intrathoracic inoculation. [a]

| Overian cycle | Stage      | Virus isolations/<br>No. pools tested | No. individuals tested |
|---------------|------------|---------------------------------------|------------------------|
| 1             | 4th instar | 0/72                                  | 1776                   |
|               | Pupae      | 0/13                                  | 254                    |
| 2             | 4th instar | 0/108                                 | 2678                   |
|               | Pupae      | 0/49                                  | 1164                   |
| Total         | 4th instar | 0/180                                 | 4454 [b]               |
|               | Pupae      | 0/62                                  | 1418 [c]               |

[a] Female mosquitoes inoculated with virus, incubated at 18 C for 7 days, fed on chicks, fed mosquitoes subsequently held for 7 days at 18 C, and then allowed to oviposit. Egg rafts pooled, hatched, and larvae reared to 4th instar or pupae at 18 C.

[b] Can reject the null hypothesis that the true "infected" population proportion,  $p=.0007$  at the  $\alpha=0.05$  level.

[c] Can reject the null hypothesis that the true "infected" population proportion,  $p=0.0021$  at the  $\alpha=0.05$  level.



Table II-7. Attempt to demonstrate transovarial transmission of Mert Park (BFN5662) virus by *Cx. tarsalis* (Knights Landing colony) following intrathoracic inoculation. [a]

| Ovarian cycle | Stage (sex) | Virus isolations/<br>No. pools tested | No. individuals tested |
|---------------|-------------|---------------------------------------|------------------------|
| 1             | 4th instar  | 0/78                                  | 1950 [b]               |
|               | Pupae       | 0/7                                   | 169 [c]                |
| 1             | Adults (F)  | 0/21                                  | 518                    |
|               | Adults (M)  | 0/17                                  | 410                    |
| 2             | Adults (F)  | 0/3                                   | 70                     |
|               | Adults (M)  | 0/3                                   | 70                     |
| Total         | Adults (F)  | 0/24                                  | 580 [d]                |
|               | Adult (M)   | 0/20                                  | 480 [e]                |

[a] Female mosquitoes inoculated with virus, incubated at 27 C for 7 days, fed on chicks, fed mosquitoes subsequently held for 7 days at 27 C, and then allowed to oviposit. Egg rafts pooled, hatched, and larvae reared to 4th instar, pupae, or adults at 27C.

[b] Can reject the null hypothesis that the true "infected" population proportion,  $p=0.0015$  at the  $\alpha=0.05$  level.

[c] Can reject the null hypothesis that the true "infected" population proportion,  $p=0.0176$  at the  $\alpha=0.05$  level.

[d] Can reject the null hypothesis that the true "infected" population proportion,  $p=0.0052$  at the  $\alpha=0.05$  level.

[e] Can reject the null hypothesis that the true "infected" population proportion,  $p=0.0062$  at the  $\alpha=0.05$  level.

Table II-8. Attempt to demonstrate transovarial transmission of Turlock (Kern 82-63) virus in Ae. epactius (Utah strain) after intrathoracic inoculation. [a]

| Ovarian cycle | Stage (sex) | Virus isolations/<br>No. pools tested | No. individuals tested |
|---------------|-------------|---------------------------------------|------------------------|
| 1             | 4th instar  | 0/13                                  | 192 [b]                |
| 1             | Adults (F)  | 0/20                                  | 329 [c]                |
| 1             | Adults (M)  | 0/5                                   | 93 [d]                 |

[a] Female mosquitoes inoculated with virus, incubated at 27 C for 7 days, fed on chicks, fed mosquitoes subsequently held for 7 days at 27 C, and then allowed to oviposit. Eggs pooled, hatched, and larvae reared to 4th instar or adults at 27 C.

[b] Can reject the null hypothesis that the true "infected" population proportion,  $p=0.01548$  at the  $\alpha=0.05$  level.

[c] Can reject the null hypothesis that the true "infected" population proportion,  $p=0.0091$  at the  $\alpha=0.05$  level.

[d] Can reject the null hypothesis that the true "infected" population proportion,  $p=0.0317$  at the  $\alpha=0.05$  level.

Table II-9. Attempt to demonstrate transovarial transmission of Hart Park (Kern 83-5350) virus in a field strain of Cx. tarsalis following intrathoracic inoculation. [a]

| Ovarian cycle | Stage (sex) | No. isolations/<br>No. pools tested | No. individuals tested |
|---------------|-------------|-------------------------------------|------------------------|
| 1             | Adult (M)   | 0/47                                | 788                    |
| 1             | Adult (F)   | 0/56                                | 941                    |
| Total         |             | 0/103                               | 1729 [b]               |

[a] Female mosquitoes inoculated with virus, incubated at 27 C for 7 days, fed on chicks, fed mosquitoes subsequently held for 5-7 days at 27 C, and then allowed to oviposit. Eggs pooled, hatched, and larvae reared to adults at 27 C.

[b] Can reject the null hypothesis that the true "infected" population proportion, p=0.0018 at the alpha=0.05 level.

Table III-1. Arboviruses known to occur in California and the results of indirect fluorescent antibody tests with hyperimmune mouse ascitic fluids from mice vaccinated against these viruses to cells infected with the 6 viruses utilized in the present survey.

| HMAF [a]             | Virus infected cells |    |     |     |     |     |
|----------------------|----------------------|----|-----|-----|-----|-----|
|                      | TUR                  | HP | SLE | WEE | GRL | LLS |
| TUR                  | + [b]                | -  | -   | -   | -   | -   |
| HP                   | -                    | +  | -   | -   | -   | -   |
| WEE                  | -                    | -  | -   | +   | -   | -   |
| SLE                  | -                    | -  | +   | -   | -   | -   |
| Gray Lodge           | -                    | -  | -   | -   | +   | -   |
| Llano Seco           | -                    | -  | -   | -   | -   | +   |
| Rio Bravo [c]        | -                    | -  | +   | -   | -   | -   |
| Powassan             | -                    | -  | +   | -   | -   | -   |
| Modoc                | -                    | -  | +   | -   | -   | -   |
| California enceph.   | -                    | -  | -   | -   | -   | -   |
| Jamestown Canyon     | -                    | -  | -   | -   | -   | -   |
| Jerry Slough         | -                    | -  | -   | -   | -   | -   |
| Buttonwillow         | -                    | -  | -   | -   | -   | -   |
| Lokern               | -                    | -  | -   | -   | -   | -   |
| Main Drain           | -                    | -  | -   | -   | -   | -   |
| Kern Canyon          | -                    | -  | -   | -   | -   | -   |
| Blue tongue (Type 8) | -                    | -  | -   | -   | -   | -   |

[a] HMAF=hyperimmune mouse ascitic fluid, diluted 1:8 in 20% beef brain in PBS-FA.

[b] The reciprocal homologous titers of the HMAFs to the 6 antigens of primary interest were as follows: TUR,  $\geq 1280$ ; HP,  $\geq 1280$ ; SLE,  $\geq 1280$ ; WEE, 640; GRL, 80; LLS, 320.

[c] The strains of viruses used were as follows: Turlock, 847-32; Hart Park, BFN5662; WEE, BFS1703; SLE, BFS1750; Llano Seco, BFN3112; Gray Lodge, BFN3187; Rio Bravo, M64; Powassan, McClain; Modoc, M544; California encephalitis, BFS283; Jamestown Canyon, 61V-2235; Jerry Slough, BFS4474; Buttonwillow, A7956; Lokern, BFS5183; Main Drain, 1966 pooled; Kern Canyon, M206; Blue tongue (type 8), ATCC VR187. See text for other viruses.

Table III-2. Summary of populations from California that were tested for antibodies to Turlock and Hart Park viruses.

| Population & purpose  | Species      | Years collected | Number of sera |
|-----------------------|--------------|-----------------|----------------|
| Diagnostic<br>CNS [a] | Human        | 1968-1982       | 1732           |
|                       | Horse        | 1967-1982       | 146            |
| Cross-sectional       | Human        | 1960            | 235            |
|                       | Horse        | 1968            | 499            |
|                       | Cattle       | 1968            | 487            |
|                       | Sheep        | 1968            | 446            |
|                       | Pig          | 1968            | 61             |
|                       | Dog          | 1968            | 44             |
|                       | Dog          | 1983            | 75             |
|                       | Feral mammal | 1982            | 208            |
|                       | Wild bird    | 1971-1973       | 163            |
| Feral mammal          | 1969-1973    | 143             |                |
| Sentinel              | Chicken      | 1978-1982       | 2715           |

[a] Obtained from the Viral and Rickettsial Disease Laboratory, California Dept. Health Services.

Table III-3. Geographical distribution by county in California of human cases of central nervous system disease tested for antibodies to Hart Park and Turlock viruses. The 1968-1976 population was tested by both hemagglutination inhibition and indirect fluorescent antibody tests (for Turlock virus) while the 1977-1982 population were tested by indirect fluorescent antibody and selectively by plaque reduction neutralization tests.

| County of residence | Year and No. tested |           |
|---------------------|---------------------|-----------|
|                     | 1968-1976           | 1977-1982 |
| Unknown or uncoded  | 49                  | 5         |
| Alameda             | 38                  | 6         |
| Butte               | 23                  | 12        |
| Calaveras           | 3                   | -         |
| Colusa              | 5                   | -         |
| Contra Costa        | 56                  | -         |
| Del Norte           | 4                   | -         |
| El Dorado           | 12                  | 4         |
| Fresno              | 65                  | 38        |
| Glenn               | 8                   | -         |
| Humboldt            | 5                   | 3         |
| Imperial            | 2                   | 12        |
| Inyo                | 2                   | 2         |
| Kern                | 122                 | 8         |
| Kings               | 7                   | 3         |
| Lake                | 5                   | 6         |
| Lassen              | 4                   | -         |
| Los Angeles         | 15                  | 116       |
| Madera              | 4                   | 1         |
| Marin               | 29                  | -         |
| Mariposa            | 2                   | -         |
| Mendocino           | 4                   | -         |
| Merced              | 11                  | 10        |
| Modoc               | 3                   | -         |
| Mono                | 1                   | -         |
| Monterey            | 28                  | -         |
| Napa                | 7                   | 4         |
| Nevada              | 3                   | -         |
| Orange              | 6                   | 1         |

Table III-3 (Cont'd)

| County of residence | 1968-1976   | 1977-1982  |
|---------------------|-------------|------------|
| Placer              | 15          | 13         |
| Riverside           | 24          | 2          |
| Sacramento          | 59          | 50         |
| San Benito          | 1           | -          |
| San Bernardino      | 27          | 1          |
| San Diego           | 3           | 134        |
| San Francisco       | 46          | 1          |
| San Joaquin         | 77          | 10         |
| San Luis Obispo     | 8           | 5          |
| San Mateo           | 64          | -          |
| Santa Barbara       | 42          | -          |
| Santa Clara         | 88          | -          |
| Santa Cruz          | 15          | 3          |
| Shasta              | 15          | 12         |
| Sierra              | -           | 7          |
| Siskiyou            | 2           | 1          |
| Solano              | 16          | -          |
| Sonoma              | 33          | 1          |
| Stanislaus          | 51          | 1          |
| Sutter              | 13          | -          |
| Tehama              | 25          | 7          |
| Tulare              | 28          | 4          |
| Tuolumne            | 3           | 1          |
| Ventura             | 13          | 2          |
| Yolo                | 32          | 11         |
| Yuba                | 4           | 6          |
| <b>Total</b>        | <b>1229</b> | <b>503</b> |

Table III-4. Geographical distribution by county in California of sera collected from horses with central nervous system disease and for antibodies to Hart Park and Turlock viruses, 1967 to 1982.

| County of residence | No. of horses tested |
|---------------------|----------------------|
| Butte               | 7                    |
| Calaveras           | 1                    |
| Colusa              | 1                    |
| Contra Costa        | 3                    |
| El Dorado           | 5                    |
| Fresno              | 7                    |
| Glenn               | 1                    |
| Humboldt            | 1                    |
| Imperial            | 3                    |
| Kern                | 27                   |
| Lassen              | 1                    |
| Los Angeles         | 4                    |
| Mendocino           | 1                    |
| Merced              | 2                    |
| Modoc               | 2                    |
| Nevada              | 1                    |
| Orange              | 2                    |
| Placer              | 3                    |
| Riverside           | 4                    |
| Sacramento          | 9                    |
| San Benito          | 1                    |
| San Bernardino      | 4                    |
| San Diego           | 4                    |
| San Joaquin         | 13                   |
| San Luis Obispo     | 1                    |
| San Mateo           | 1                    |
| Santa Barbara       | 1                    |
| Santa Clara         | 1                    |
| Shasta              | 3                    |
| Solano              | 2                    |
| Sonoma              | 6                    |
| Stanislaus          | 5                    |
| Tehama              | 3                    |
| Yolo                | 12                   |
| Yuba                | 3                    |
| Unknown             | 1                    |
| <b>Total</b>        | <b>146</b>           |



Table III-5. Geographical distribution by county of sera collected from domestic mammals and tested for antibodies by the indirect fluorescence method to Hart Park and Turlock viruses, 1968.

| County<br>of<br>residence | Species and No. tested |            |            |           |           |             |
|---------------------------|------------------------|------------|------------|-----------|-----------|-------------|
|                           | Cattle                 | Horse      | Sheep      | Pig       | Dog       | Total       |
| Anador                    | 0                      | 0          | 35         | 0         | 0         | 35          |
| Butte                     | 21                     | 0          | 10         | 0         | 0         | 31          |
| Fresno                    | 51                     | 80         | 86         | 0         | 6         | 223         |
| Glenn                     | 22                     | 81         | 30         | 2         | 2         | 137         |
| Kings                     | 0                      | 20         | 0          | 10        | 0         | 30          |
| Lassen                    | 38                     | 44         | 33         | 0         | 8         | 123         |
| Modoc                     | 5                      | 9          | 19         | 0         | 0         | 33          |
| Placer                    | 0                      | 0          | 11         | 0         | 0         | 11          |
| Riverside                 | 8                      | 14         | 0          | 0         | 0         | 22          |
| San Joaquin               | 80                     | 60         | 38         | 20        | 2         | 200         |
| Solano                    | 0                      | 0          | 9          | 6         | 0         | 15          |
| Stanislaus                | 51                     | 33         | 38         | 10        | 2         | 134         |
| Tehama                    | 56                     | 49         | 48         | 0         | 8         | 161         |
| Tulare                    | 50                     | 0          | 8          | 3         | 0         | 61          |
| Yolo                      | 105                    | 107        | 71         | 10        | 16        | 309         |
| <b>Total</b>              | <b>487</b>             | <b>497</b> | <b>436</b> | <b>61</b> | <b>44</b> | <b>1533</b> |

Table III-6. Geographical distribution by county of sera from the wild mammals tested for antibodies to Hart Park and Turlock viruses, 1982-1983.

| County<br>of<br>residence | Species and No. tested |           |            |          |             |           |
|---------------------------|------------------------|-----------|------------|----------|-------------|-----------|
|                           | Coyote                 | Bobcat    | Kit<br>Fox | Skunk    | Gray<br>Fox | Other [a] |
| Alameda                   | 7                      | -         | -          | -        | -           | -         |
| Amador                    | 9                      | 11        | -          | -        | -           | 1         |
| Butte                     | 7                      | -         | -          | -        | -           | -         |
| Lassen                    | 4                      | 1         | -          | -        | -           | -         |
| Los Angeles               | 26                     | -         | -          | -        | -           | -         |
| Mariposa                  | -                      | -         | -          | -        | 1           | 1         |
| Mendocino                 | 11                     | -         | -          | -        | -           | -         |
| Modoc                     | 9                      | -         | -          | -        | -           | -         |
| Monterey                  | 13                     | 30        | 1          | 4        | 5           | 42        |
| San Diego                 | 3                      | -         | -          | -        | -           | -         |
| San Luis Obispo           | 5                      | -         | -          | -        | -           | -         |
| Santa Barbara             | 1                      | -         | -          | -        | -           | -         |
| Siskiyou                  | 14                     | -         | -          | -        | -           | 2         |
| <b>Total</b>              | <b>109</b>             | <b>42</b> | <b>1</b>   | <b>7</b> | <b>11</b>   | <b>38</b> |

[a] Others included 3 badgers, 8 cottontail rabbits, 26 tree squirrels, and 1 black bear.

Table III-7. Geographical distribution by county in California of sera from sentinel chicken flocks used in the arbovirus surveillance program, 1978-1982.

| County<br>of<br>residence | Year and No. tested |            |            |            |            | Total       |
|---------------------------|---------------------|------------|------------|------------|------------|-------------|
|                           | 1978                | 1979       | 1980       | 1981       | 1982       |             |
| Butte                     | 85                  | 89         | 94         | 43         | 45         | 356         |
| Colusa                    | -                   | 47         | 22         | 23         | -          | 92          |
| Fresno                    | -                   | 18         | 13         | 24         | 36         | 91          |
| Glenn                     | 48                  | 24         | 24         | -          | 23         | 119         |
| Imperial                  | -                   | 19         | 16         | 18         | 39         | 92          |
| Kern                      | 194                 | 177        | 212        | 114        | 80         | 777         |
| Kings                     | -                   | -          | -          | -          | 24         | 24          |
| Merced                    | -                   | -          | -          | 23         | 24         | 47          |
| Placer                    | 25                  | 25         | 25         | -          | -          | 75          |
| Riverside                 | -                   | 25         | 33         | 37         | 33         | 128         |
| Sacramento                | -                   | -          | -          | 24         | 22         | 46          |
| San Bernardino            | -                   | 22         | -          | 19         | -          | 41          |
| San Diego                 | -                   | 24         | 23         | -          | -          | 47          |
| San Joaquin               | -                   | -          | -          | 24         | 22         | 46          |
| Shasta                    | -                   | 43         | 46         | 22         | 23         | 134         |
| Tehama                    | -                   | -          | 24         | 21         | 23         | 68          |
| Tulare                    | -                   | 6          | 24         | 43         | 47         | 120         |
| Yuba/Sutter               | 112                 | 103        | 104        | 46         | 47         | 412         |
| <b>Total</b>              | <b>464</b>          | <b>622</b> | <b>660</b> | <b>481</b> | <b>488</b> | <b>2715</b> |

Table III-8. Comparison of Hart Park plaque reduction neutralization (PRN) and indirect fluorescent antibody (IFA) test results on the convalescent sera from human cases of central nervous system disease, 1979-1981.

| IFA     | PRN     |         | Total |
|---------|---------|---------|-------|
|         | No. neg | No. pos |       |
| No. neg | 223     | 13      | 236   |
| No. pos | 0       | 0       | 0     |
| Total   | 223     | 13      | 236   |

Table III-9. Results of Hart Park virus plaque reduction neutralization (PRN) tests on the paired sera from human cases of central nervous system disease, 1979-1981, whose convalescent sample neutralized Hart Park virus at a dilution of 1:10.

| Serum No. | 80% PRN titer |
|-----------|---------------|
| 103060    | <20 [a]       |
| 103737    | 40            |
| 103988    | <20           |
| 103989    | 80            |
| 103921    | 160           |
| 104363    | 160           |
| 202001    | 40            |
| 202002    | 160           |
| 203036    | <20           |
| 203037    | 40            |
| 203038    | 80            |
| 203039    | 80            |
| 203040    | <20           |
| 203041    | <20           |
| 203069    | 20            |
| 203070    | 160           |
| 203071    | 40            |
| 203072    | 80            |
| 103021    | <20           |
| 103022    | 80            |
| 003017    | <20           |
| 003018    | 20            |
| 003055    | <20           |
| 003056    | <20           |
| 003075    | 40            |
| 003076    | 40            |

[a] Reciprocal of antibody dilution.

Table III-10. Comparison of hemagglutination inhibition (HI) and indirect fluorescent antibody (IFA) test results for Turlock antibodies with convalescent sera from human central nervous system cases, 1968 to 1976 [a].

| IFA     | HI      |         | Total |
|---------|---------|---------|-------|
|         | No. neg | No. pos |       |
| No. neg | 1227    | 1       | 1228  |
| No. pos | 1 [a]   | 0       | 1     |
| Total   | 1228    | 1       | 1229  |

[a] The pair in which the convalescent member was IFA positive for TUR virus did not show rising titers when the acute and convalescent sera were examined simultaneously by either indirect fluorescent antibody or PRN test.

Table III-11. Comparison of hemagglutination inhibition (HI) and indirect fluorescent antibody (IFA) test results for western equine encephalomyelitis antibodies with convalescent sera from human central nervous system cases, 1968 to 1976.

| IFA     | HI      |         | Total |
|---------|---------|---------|-------|
|         | No. neg | No. pos |       |
| No. neg | 1189    | 6       | 1195  |
| No. pos | 6       | 28      | 34    |
| Total   | 1195    | 34      | 1229  |

Table III-12. Comparison of indirect fluorescent antibody (IFA) and hemagglutination inhibition (HI) test results for St. Louis encephalitis antibodies on the convalescent sera from human central nervous system cases, 1968-1976.

| IFA     | HI      |         | Total |
|---------|---------|---------|-------|
|         | No. neg | No. pos |       |
| No. neg | 1099    | 28      | 1127  |
| No. pos | 21      | 81      | 102   |
| Total   | 1120    | 109     | 1229  |



Table III-13. Indirect fluorescent antibody (IFA) test results for Turlock and Hart Park antibody in the convalescent sera from central nervous system disease cases in horses, 1967-1982.

| Virus | IFA     |         |       |
|-------|---------|---------|-------|
|       | No. neg | No. pos | Total |
| HP    | 143     | 3       | 146   |
| TUR   | 138     | 8       | 146   |

[a] None of the pairs in which the convalescent member was IFA test positive for either HP or TUR virus showed rising titers when the acute and convalescent sera were examined simultaneously.

Table III-14. Test results on sera from domestic mammals for Turlock and Hart Park antibodies by the indirect fluorescence (IFA) method.

| Species       | IFA     |         | Total |
|---------------|---------|---------|-------|
|               | No. neg | No. pos |       |
| <b>Cattle</b> |         |         |       |
| HP            | 487     | 0       | 487   |
| TUR           | 487     | 0       | 487   |
| <b>Horse</b>  |         |         |       |
| HP            | 497     | 2       | 499   |
| TUR           | 374     | 125     | 499   |
| <b>Sheep</b>  |         |         |       |
| HP            | 446     | 0       | 446   |
| TUR           | 445     | 1       | 446   |
| <b>Pig</b>    |         |         |       |
| HP            | 61      | 0       | 61    |
| TUR           | 56      | 5       | 61    |
| <b>Goat</b>   |         |         |       |
| HP            | 8       | 0       | 8     |
| TUR           | 8       | 0       | 8     |
| <b>Dog</b>    |         |         |       |
| HP            | 28      | 16      | 44    |
| TUR           | 31      | 13      | 44    |

Table III-15. Comparison of the indirect fluorescent antibody (IFA) and plaque reduction neutralization (PRN) test results for detection of Hart Park antibodies in a cross-sectional sample of domestic mammals.

| Species<br>IFA   | PRN        |         |         | Total |
|------------------|------------|---------|---------|-------|
|                  | Not tested | No. neg | No. pos |       |
| <b>Cattle</b>    |            |         |         |       |
| No. neg          | 454        | 31      | 2       | 487   |
| No. pos          | 0          | 0       | 0       | 0     |
| Total            | 454        | 31      | 2       | 487   |
| <b>Horse</b>     |            |         |         |       |
| No. neg          | 400        | 84      | 13      | 497   |
| No. pos          | 2          | 0       | 0       | 0     |
| Total            | 402        | 84      | 13      | 497   |
| <b>Sheep</b>     |            |         |         |       |
| No. neg          | 396        | 50      | 0       | 446   |
| No. pos          | 0          | 0       | 0       | 0     |
| Total            | 396        | 50      | 0       | 446   |
| <b>Pig</b>       |            |         |         |       |
| No. neg          | 0          | 50      | 11      | 61    |
| No. pos          | 0          | 0       | 0       | 0     |
| Total            | 0          | 50      | 11      | 61    |
| <b>Dog</b>       |            |         |         |       |
| No. neg          | 1          | 8       | 19      | 28    |
| No. pos          | 0          | 0       | 16      | 16    |
| Total            | 1          | 8       | 35      | 44    |
| <b>Total [a]</b> |            |         |         |       |
| No. neg          | 1251       | 230     | 46      | 1227  |
| Postive          | 2          | 0       | 16      | 18    |
| Total            | 1253       | 230     | 62      | 1547  |

[a] Goats were also tested by both IFA and PRN tests for HP, 1 of 8 was positive by PRN test while none were positive by IFA test.

Table III-16. Comparison of the indirect fluorescent antibody (IFA) and hemagglutination inhibition (HI) test results for detection of Turlock antibodies in sera from domestic mammals.

| Species<br>HI    | IFA         |            |             |
|------------------|-------------|------------|-------------|
|                  | No. neg     | No. pos    | Total       |
| <b>Cattle</b>    |             |            |             |
| No. neg          | 475         | 0          | 475         |
| No. pos          | 12          | 0          | 12          |
| <b>Total</b>     | <b>487</b>  | <b>0</b>   | <b>487</b>  |
| <b>Horse</b>     |             |            |             |
| No. neg          | 322         | 45         | 367         |
| No. pos          | 52          | 80         | 132         |
| <b>Total</b>     | <b>374</b>  | <b>125</b> | <b>499</b>  |
| <b>Sheep</b>     |             |            |             |
| No. neg          | 436         | 0          | 436         |
| No. pos          | 9           | 1          | 10          |
| <b>Total</b>     | <b>445</b>  | <b>1</b>   | <b>446</b>  |
| <b>Pig</b>       |             |            |             |
| No. neg          | 55          | 1          | 56          |
| No. pos          | 1           | 4          | 5           |
| <b>Total</b>     | <b>56</b>   | <b>5</b>   | <b>61</b>   |
| <b>Dog</b>       |             |            |             |
| No. neg          | 29          | 4          | 33          |
| No. pos          | 2           | 9          | 11          |
| <b>Total</b>     | <b>31</b>   | <b>13</b>  | <b>44</b>   |
| <b>Total [a]</b> |             |            |             |
| No. neg          | 1325        | 50         | 1361        |
| No. pos          | 76          | 94         | 176         |
| <b>Total</b>     | <b>1401</b> | <b>144</b> | <b>1545</b> |

[a] Total includes 8 goats, all were negative by both HI and IFA tests.

Table III-17. Comparison of results of indirect fluorescent antibody (IFA) and hemagglutination inhibition (HI) tests, controlling for plaque reduction neutralization (PRN), for detection of Turlock antibodies in sera from domestic mammals.

| PRN        | HI      | IFA     |         | Total |
|------------|---------|---------|---------|-------|
|            |         | No. neg | No. pos |       |
| Not Tested | No. neg | 1113    | 19      | 1142  |
|            | No. pos | 62      | 59      | 121   |
|            | Total   | 1175    | 78      | 1253  |
| Negative   | No. neg | 191     | 7       | 198   |
|            | No. pos | 6       | 1       | 7     |
|            | Total   | 197     | 8       | 205   |
| Positive   | No. neg | 17      | 22      | 39    |
|            | No. pos | 12      | 36      | 48    |
|            | Total   | 29      | 58      | 87    |

Table III-18. Comparison of the indirect fluorescent antibody (IFA) and hemagglutination inhibition (HI) test results for detection of western equine encephalomyelitis antibodies in sera from domestic mammals.

| Species<br>HI    | IFA         |            |             |
|------------------|-------------|------------|-------------|
|                  | No. neg     | No. pos    | Total       |
| <b>Cattle</b>    |             |            |             |
| No. neg          | 461         | 1          | 462         |
| No. pos          | 25          | 0          | 25          |
| <b>Total</b>     | <b>486</b>  | <b>1</b>   | <b>487</b>  |
| <b>Horse</b>     |             |            |             |
| No. neg          | 88          | 11         | 99          |
| No. pos          | 77          | 323        | 400         |
| <b>Total</b>     | <b>165</b>  | <b>334</b> | <b>499</b>  |
| <b>Sheep</b>     |             |            |             |
| No. neg          | 411         | 3          | 414         |
| No. pos          | 31          | 1          | 32          |
| <b>Total</b>     | <b>442</b>  | <b>4</b>   | <b>446</b>  |
| <b>Pig</b>       |             |            |             |
| No. neg          | 56          | 1          | 57          |
| No. pos          | 1           | 3          | 4           |
| <b>Total</b>     | <b>57</b>   | <b>4</b>   | <b>61</b>   |
| <b>Dog</b>       |             |            |             |
| No. neg          | 28          | 4          | 32          |
| No. pos          | 3           | 8          | 11          |
| <b>Total</b>     | <b>31</b>   | <b>12</b>  | <b>43</b>   |
| <b>Total [a]</b> |             |            |             |
| No. neg          | 1052        | 20         | 1072        |
| No. pos          | 137         | 335        | 472         |
| <b>Total</b>     | <b>1189</b> | <b>355</b> | <b>1544</b> |

[a] Total includes 8 goats, all were negative by both HI and IFA tests.

Table III-19. Comparison of the indirect fluorescent antibody (IFA) and hemagglutination inhibition (HI) test results for detection of St. Louis encephalitis antibodies in sera from domestic mammals.

| Species<br>HI    | IFA         |            |             |
|------------------|-------------|------------|-------------|
|                  | No. neg     | No. pos    | Total       |
| <b>Cattle</b>    |             |            |             |
| No. neg          | 457         | 11         | 468         |
| No. pos          | 18          | 1          | 19          |
| <b>Total</b>     | <b>475</b>  | <b>12</b>  | <b>487</b>  |
| <b>Horse</b>     |             |            |             |
| No. neg          | 311         | 7          | 318         |
| No. pos          | 116         | 65         | 181         |
| <b>Total</b>     | <b>427</b>  | <b>72</b>  | <b>499</b>  |
| <b>Sheep</b>     |             |            |             |
| No. neg          | 428         | 5          | 433         |
| No. pos          | 12          | 1          | 13          |
| <b>Total</b>     | <b>440</b>  | <b>6</b>   | <b>446</b>  |
| <b>Pig</b>       |             |            |             |
| No. neg          | 48          | 5          | 53          |
| No. pos          | 2           | 6          | 8           |
| <b>Total</b>     | <b>50</b>   | <b>11</b>  | <b>61</b>   |
| <b>Dog</b>       |             |            |             |
| No. neg          | 25          | 7          | 32          |
| No. pos          | 0           | 11         | 11          |
| <b>Total</b>     | <b>25</b>   | <b>18</b>  | <b>43</b>   |
| <b>Total (a)</b> |             |            |             |
| No. neg          | 1276        | 36         | 1312        |
| No. pos          | 148         | 84         | 232         |
| <b>Total</b>     | <b>1424</b> | <b>120</b> | <b>1544</b> |

(a) Total includes 8 goats of which one was positive by the IFA test and negative by the HI, the other 7 were negative by both tests.

Table III-20. Distribution of Turlock antibody titers by the indirect fluorescence method among animals screened and found positive at a dilution of 1:8.

| Titer [a]    | Species and No. tested |           |           |
|--------------|------------------------|-----------|-----------|
|              | Pig                    | Dog       | Horse     |
| 1:8          | 0                      | 0         | 3         |
| 1:16         | 3                      | 1         | 10        |
| 1:32         | 1                      | 3         | 17        |
| 1:64         | 1                      | 2         | 10        |
| 1:128        | 0                      | 3         | 0         |
| ≥1:256       | 0                      | 4         | 1         |
| <b>Total</b> | <b>5</b>               | <b>13</b> | <b>41</b> |

[a] All positives for porcine and canine were titered, a random sample of the screen positive samples was chosen from among the Horses for titration.



Table III-21. Summary of wild mammals tested for Hart Park and Turlock plaque reduction neutralization (PRN) antibodies.

| Species              | PRN     |         | Total |
|----------------------|---------|---------|-------|
|                      | No. neg | No. pos |       |
| Coyote               | 109     | 0       | 109   |
| Bobcat               | 42      | 0       | 42    |
| Kit Fox              | 1       | 0       | 1     |
| Gray Fox             | 11      | 0       | 11    |
| Badger               | 3       | 0       | 3     |
| Skunk                | 7       | 0       | 7     |
| Squirrel             | 26      | 0       | 26    |
| Cottontail<br>rabbit | 8       | 0       | 8     |
| Black Bear           | 1       | 0       | 1     |

Table III-22. Plaque reduction neutralization (PRN) test results for Hart Park and Turlock antibodies on sera from military working (MWD) and household dogs collected in California during 1983.

| Population | PRN     |         |           |
|------------|---------|---------|-----------|
|            | No. neg | No. pos | Total [a] |
| <b>HP</b>  |         |         |           |
| MWD [b]    | 36      | 5       | 41        |
| Pets [c]   | 31      | 5       | 36        |
| Total      | 67      | 10      | 77        |
| <b>TUR</b> |         |         |           |
| MWD        | 30      | 9       | 39        |
| Pets       | 34      | 2       | 36        |
| Total      | 64      | 11      | 75        |

[a] Discrepancy in totals because of toxicity of some sera to DECC cells used in TUR PRN test.

[b] MWD came from the following locations: Mather AFB (13), Sacramento County; Castle AFB (6) Merced County; Travis AFB (6), Solano County; S. F. Bay Area (7); Vandenberg AFB (10), Santa Barbara County.

[c] Pets were all from Bakersfield, CA.

Table III-23. Plaque reduction neutralization test results for Hart Park and Turlock antibodies on sera from small mammals collected in Kern County, 1969-1971.

| Species                       | HP      |         | TUR     |         |
|-------------------------------|---------|---------|---------|---------|
|                               | No. pos | No. neg | No. pos | No. neg |
| Kangaroo rat<br>(San Joaquin) | 1       | 34 [a]  | 0       | 35      |
| Kangaroo rat                  | 0       | 1       | 0       | 1       |
| Antelope ground<br>squirrel   | 3       | 29 [a]  | 1       | 31 [a]  |
| White footed<br>mouse         | 0       | 15      | 0       | 13      |
| Cottontail<br>rabbit          | 0       | 2       | 0       | 2       |
| Grasshopper<br>mouse          | 0       | 2       | 0       | 2       |
| Jackrabbit                    | 0       | 32      | 0       | 32      |
| House mouse                   | 0       | 1       | 0       | 1       |
| California<br>ground squirrel | 0       | 10      | 0       | 9       |
| Harvest mouse                 | 0       | 2       | 0       | 2       |
| Flying squirrel               | 0       | 1       | 0       | 1       |

[a] All positives from Lerdo area (Lerdo Grid or Lerdo Bridge).

Table III-24. Plaque reduction neutralization test results for antibodies to Turlock and Hart Park viruses in the sera from wild birds collected in Kern County, 1971-1973.

| Species           | HP      |         | TUR     |         |
|-------------------|---------|---------|---------|---------|
|                   | No. pos | No. neg | No. pos | No. neg |
| House finch [a]   | 53      | 23      | 33      | 32      |
| House sparrow [b] | 0       | 79      | 0       | 76      |

[a] House finches from the following locations: C. Tracy Ranch, 73; South Belridge Park, 3. All positives from C. Tracy Ranch.

[b] House sparrows from the following locations: C. Tracy ranch, 6; Lost hills, 27; North Belridge, 7; Lerdo Highway, 18; South Belridge Park, 18.

Table III-25. Comparison of results of the indirect fluorescent antibody (IFA) and plaque reduction neutralization (PRN) tests for detection of Turlock antibodies in sera from sentinel chickens.

| IFA     | PRN     |         | Total |
|---------|---------|---------|-------|
|         | No. neg | No. pos |       |
| 1978    |         |         |       |
| No. neg | 323     | 13      | 336   |
| No. pos | 10      | 113     | 123   |
| Total   | 333     | 126     | 459   |
| 1979    |         |         |       |
| No. neg | 416     | 33      | 449   |
| No. pos | 8       | 154     | 162   |
| Total   | 424     | 187     | 611   |
| Total   |         |         |       |
| No. neg | 739     | 46      | 785   |
| No. pos | 18      | 267     | 285   |
| Total   | 757     | 313     | 1070  |

Relative specificity:  $739/757 = .9762$

Relative sensitivity:  $267/313 = .8350$

Table III-26. Comparison of results of the indirect fluorescent antibody (IFA) and plaque reduction neutralization (PRN) tests for detection of St. Louis encephalitis antibodies in sera from sentinel chickens.

| IFA      | PRN     |         |       |
|----------|---------|---------|-------|
|          | No. neg | No. pos | Total |
| -----    |         |         |       |
| 1978     |         |         |       |
| No. neg  | 459     | 0       | 459   |
| No. pos  | 0       | 0       | 0     |
| Total    | 459     | 0       | 459   |
| 1979     |         |         |       |
| No. neg  | 601     | 1       | 602   |
| No. pos  | 4       | 5       | 9     |
| Total    | 605     | 6       | 611   |
| -----    |         |         |       |
| Total    |         |         |       |
| No. neg  | 1060    | 1       | 1061  |
| Positive | 4       | 5       | 9     |
| Total    | 1064    | 6       | 1070  |
| -----    |         |         |       |

Relative specificity:  $1060/1064 = .9962$

Relative sensitivity:  $5/6 = .8333$

Table III-27. Comparison of results of the indirect fluorescent antibody (IFA) and plaque reduction neutralization (PRN) tests for detection of western equine encephalomyelitis antibodies in sera from sentinel chickens.

| IFA     | PRN     |         | Total |
|---------|---------|---------|-------|
|         | No. neg | No. pos |       |
| 1978    |         |         |       |
| No. neg | 332     | 5       | 337   |
| No. pos | 2       | 120     | 122   |
| Total   | 334     | 125     | 459   |
| 1979    |         |         |       |
| No. neg | 362     | 6       | 368   |
| No. pos | 3       | 240     | 243   |
| Total   | 365     | 246     | 611   |
| Total   |         |         |       |
| No. neg | 694     | 11      | 705   |
| No. pos | 5       | 360     | 365   |
| Total   | 699     | 371     | 1070  |

Relative specificity:  $694/699 = .9928$

Relative sensitivity:  $360/371 = .9704$

Table III-28. Comparison of results of the micro- and standard-indirect fluorescent antibody (IFA) tests for detection of St. Louis encephalitis antibodies in sera from sentinel chickens.

| Micro-IFA | Standard-IFA |         | Total |
|-----------|--------------|---------|-------|
|           | No. neg      | No. pos |       |
| 1979      |              |         |       |
| No. neg   | 598          | 4       | 602   |
| No. pos   | 4            | 5       | 9     |
| Total     | 602          | 9       | 611   |
| 1980      |              |         |       |
| No. neg   | 522          | 0       | 522   |
| No. pos   | 3            | 2       | 5     |
| Total     | 525          | 2       | 527   |
| 1981      |              |         |       |
| No. neg   | 468          | 1       | 469   |
| No. pos   | 5            | 7       | 12    |
| Total     | 473          | 8       | 481   |
| 1982      |              |         |       |
| No. neg   | 656          | 0       | 656   |
| No. pos   | 2            | 0       | 2     |
| Total     | 658          | 0       | 658   |
| Total     |              |         |       |
| No. neg   | 2244         | 5       | 2249  |
| No. pos   | 14           | 14      | 28    |
| Total     | 2258         | 19      | 2277  |

Relative specificity:  $2244/2258 = .9938$

Relative sensitivity:  $14/19 = .7376$



Table III-29. Comparison of results of the micro- and standard-indirect fluorescent antibody (IFA) tests for detection of western equine encephalomyelitis antibodies in sera from sentinel chickens.

|         | Standard-IFA |         | Total |
|---------|--------------|---------|-------|
|         | No. neg      | No. pos |       |
| -----   |              |         |       |
| 1979    |              |         |       |
| No. neg | 362          | 6       | 368   |
| No. pos | 10           | 233     | 243   |
| Total   | 372          | 239     | 622   |
| 1980    |              |         |       |
| No. neg | 377          | 1       | 378   |
| No. pos | 7            | 142     | 149   |
| Total   | 384          | 143     | 517   |
| 1981    |              |         |       |
| No. neg | 436          | 3       | 439   |
| No. pos | 3            | 39      | 42    |
| Total   | 439          | 42      | 481   |
| 1982    |              |         |       |
| No. neg | 555          | 3       | 558   |
| No. pos | 4            | 96      | 100   |
| Total   | 559          | 99      | 658   |
| -----   |              |         |       |
| Total   |              |         |       |
| No. neg | 1730         | 13      | 1743  |
| No. pos | 24           | 510     | 534   |
| Total   | 1754         | 523     | 2227  |
| -----   |              |         |       |

Specificity:  $1730/1754 = .9863$

Sensitivity:  $510/523 = .9751$

Table IV-1. Number of rural sentinel chickens used in the surveillance program from 1978-1982.

| County        | Year and number of birds |            |            |            |            | Total       |
|---------------|--------------------------|------------|------------|------------|------------|-------------|
|               | 1978                     | 1979       | 1980       | 1981       | 1982       |             |
| Butte         | 45                       | 45         | 45         | 19         | 21         | 175         |
| Colusa        | -                        | 47         | 22         | 23         | -          | 92          |
| Fresno        | -                        | 18         | 13         | 24         | 36         | 91          |
| Glenn         | 48                       | -          | -          | -          | 23         | 71          |
| Imperial      | -                        | 19         | 16         | 18         | 39         | 92          |
| Kern          | 182                      | 156        | 191        | 91         | 80         | 700         |
| Kings         | -                        | -          | -          | -          | 24         | 24          |
| Merced        | -                        | -          | -          | 23         | 24         | 47          |
| Placer        | 25                       | 25         | 25         | -          | -          | 75          |
| Riverside     | -                        | 25         | 33         | 37         | 33         | 128         |
| Sacramento    | -                        | -          | -          | 24         | 22         | 46          |
| San Berardino | -                        | 22         | -          | 19         | -          | 41          |
| San Diego     | -                        | 24         | 23         | -          | -          | 47          |
| San Joaquin   | -                        | -          | -          | 24         | 22         | 67          |
| Shasta        | -                        | 43         | 46         | 22         | 23         | 134         |
| Tehama        | -                        | -          | 24         | 21         | 23         | 68          |
| Tulare        | -                        | 6          | 24         | 43         | 47         | 120         |
| Yuba/Sutter   | 89                       | 85         | 80         | 23         | 23         | 300         |
| <b>Total</b>  | <b>389</b>               | <b>515</b> | <b>542</b> | <b>411</b> | <b>461</b> | <b>2318</b> |

Table IV-2. Variables used in analyses.

| Variable | Description  |
|----------|--|
| LTI      | Geometric mean number of <u>Cx. tarsalis</u> collected per light trap night in each county<br>[Sum log ( <u>Cx. tarsalis</u> +1)/number light traps]<br>10 |
| MIR      | Number of viral isolates (HP, TUR, or WEE) per 1000 <u>Cx. tarsalis</u> tested<br>[No. isolates/No. mosquitoes tested X 1000]                              |
| *CHIK    | Percent of susceptible chickens acquiring antibody during month<br>[No. new positives/No. susceptible chickens X 100]                                      |
| TMEAN    | Monthly mean temperature<br>[Sum(daily high + daily low/2)/Days in month]  |
| TO       | Number of degree days over 5.3 C in the current month<br>[Sum(TMEAN - 5.3 C)]  |
| TOTTO    | Cumulative number of degree days over 5.3 C for the year<br>[Sum (TOO through current month)]  |
| TMAX     | Number of degree days over 34 C in the current month<br>[Sum(daily high - 34 C)]   |
| TOTMAX   | Cumulative number of degree days over 34 C for the year<br>[Sum (TMAX) through current month]  |
| RAIN     | Rainfall (mm) in the current month<br>[Sum (daily rainfall)]   |
| TOTRAIN  | Cumulative rainfall (mm) for the year<br>[Sum (RAIN) through the current month]  |
| RVRFLOW  | Volume (acre-feet) flowing past Stockdale Highway in Kern County (Used in Kern analysis only)  |
| HRSLITE  | Number of hours from sunup to sundown at midmonth  |
| CHMNO    | Change in number of hours light from midmonth previous to midmonth current month<br>[HRSLITE(prev month) - HRSLITE(current month)]                         |
| CHBEN    | Change in number of hours light from beginning to end of current month<br>[No. hours light at beginning of month - No. hours at end]                       |

Table IV-3. Annual statewide statistics for viral isolations from female Cx. tarsalis and acquisition of antibody by rural sentinel chickens, 1978-1982.

| Year | No. female<br><u>Cx.</u><br><u>tarsalis</u><br>tested | MIR [a] |      |      | Chicken [b]<br>seroconversions |      |
|------|---|---------|------|------|--------------------------------|------|
|      |   | HP      | TUR  | WEE  | TUR                            | WEE  |
| 1978 | 65,918  | 0.33    | 0.49 | 1.21 | 5.21                           | 5.01 |
| 1979 | 86,676  | 0.13    | 0.57 | 1.31 | 5.48                           | 6.18 |
| 1980 | 150,484   | 0.48    | 0.30 | 0.41 | 3.57                           | 2.94 |
| 1981 | 94,065  | 1.04    | 0.69 | 0.51 | 5.18                           | 1.67 |
| 1982 | 104,208   | 1.02    | 1.18 | 1.98 | 7.73                           | 5.87 |
| All  | 501,351   | 0.62    | 0.63 | 1.02 | 5.34                           | 4.29 |

[a] MIR=Minimum infection rate/1000 Cx. tarsalis.

[b] Weighted mean monthly seroconversion (%) for each year.

Table IV-4. Monthly statewide statistics for viral isolations from female Cx. tarsalis and acquisition of antibody by rural sentinel chickens, 1978-1982.

| Month | No. female<br><u>Cx.</u><br><u>tarsalis</u><br>tested | MIR [a] |      |      | Chicken [b]<br>seroconversions |      |
|-------|---|---------|------|------|--------------------------------|------|
|       |   | HP      | TUR  | WEE  | TUR                            | WEE  |
| JAN   | 338   | 0.00    | 0.00 | 2.96 | -                              | -    |
| FEB   | 46  | 0.00    | 0.00 | 0.00 | -                              | -    |
| MAR   | 817   | 0.00    | 1.22 | 0.00 | -                              | -    |
| APR   | 12,188  | 0.08    | 0.16 | 0.00 | -                              | -    |
| MAY   | 36,656  | 0.33    | 0.79 | 0.16 | 0.26                           | 0.13 |
| JUN   | 73,764  | 0.98    | 0.84 | 1.59 | 1.87                           | 1.43 |
| JUL   | 135,264   | 1.00    | 0.72 | 0.46 | 5.80                           | 5.77 |
| AUG   | 150,849   | 0.53    | 0.58 | 1.27 | 12.35                          | 8.69 |
| SEP   | 81,186  | 0.10    | 0.41 | 1.55 | 11.00                          | 9.10 |
| OCT   | 10,205  | 0.10    | 0.20 | 0.69 | 1.22                           | 0.85 |
| NOV   | 37  | 0.00    | 0.00 | 0.00 | -                              | -    |
| DEC   | 1   | 0.00    | 0.00 | 0.00 | -                              | -    |
| ALL   | 501,351   | 0.62    | 0.63 | 1.02 | 5.34                           | 4.29 |

[a] MIR=Minimum infection rate/1000 Cx. tarsalis.

[b] Weighted mean monthly seroconversion (%) for the 5 year period.

Table IV-5. Five year cumulative statistics in California counties for viral isolations from female Cx. tarsalis and acquisition of antibody by rural sentinel chickens, 1978-1982.

| County       | No. female<br><u>Cx.</u><br><u>tarsalis</u><br>tested | MIR (a) |      |      | Chicken (b)<br>seroconversions |       |
|--------------|---|---------|------|------|--------------------------------|-------|
|              |   | HP      | TUR  | WEE  | TUR                            | WEE   |
| Alameda      | 71  | 14.08   | 0.0  | 0.0  | -                              | -     |
| Butte        | 36,761  | 0.24    | 0.19 | 0.33 | 7.05                           | 3.22  |
| Colusa       | 12,615  | 0.24    | 0.24 | 0.48 | 5.36                           | 5.37  |
| Contra Costa | 452   | 0.0     | 0.0  | 0.0  | -                              | -     |
| Fresno       | 3,666   | 0.27    | 0.27 | 0.0  | 5.05                           | 4.64  |
| Glenn        | 10,462  | 0.29    | 0.39 | 0.10 | 4.90                           | 7.51  |
| Humboldt     | 62  | 0.0     | 0.0  | 0.0  | -                              | -     |
| Imperial     | 30,399  | 0.0     | 0.46 | 2.34 | 3.08                           | 15.18 |
| Inyo         | 3,879   | 0.0     | 0.52 | 0.0  | -                              | -     |
| Kern         | 127,340   | 1.34    | 0.93 | 1.23 | 6.83                           | 5.87  |
| Kings        | 2,503   | 0.40    | 1.20 | 2.40 | 2.90                           | 17.76 |
| Lake         | 162   | 0.0     | 0.0  | 0.0  | -                              | -     |
| Los Angeles  | 226   | 0.0     | 0.0  | 0.0  | -                              | -     |
| Madera       | 1,471   | 0.0     | 0.68 | 1.36 | -                              | -     |
| Marin-Sonoma | 1,937   | 0.0     | 0.0  | 0.0  | -                              | -     |
| Mendocino    | 18  | 0.0     | 0.0  | 0.0  | -                              | -     |
| Merced       | 6,005   | 0.33    | 0.33 | 0.0  | -                              | -     |
| Napa         | 225   | 4.44    | 0.0  | 0.0  | -                              | -     |

Table IV-5. (cont'd)

| County          | No. tested | HP   | TUR  | WEE  | TUR   | WEE  |
|-----------------|------------|------|------|------|-------|------|
| Orange          | 1,025      | 0.0  | 1.95 | 0.0  | -     | -    |
| Placer          | 10,212     | 0.49 | 0.49 | 0.20 | 4.90  | 4.76 |
| Riverside       | 66,517     | 0.12 | 0.42 | 1.28 | 2.95  | 1.21 |
| Sacramento-Yolo | 29,884     | 0.17 | 0.27 | 0.23 | 2.64  | 0.0  |
| San Bernardino  | 20,755     | 0.05 | 1.40 | 2.40 | 0.82  | 0.41 |
| San Diego       | 1,628      | 2.45 | 1.22 | 0.0  | 0.0   | 0.0  |
| San Joaquin     | 5,755      | 0.35 | 0.35 | 0.17 | 10.62 | 0.0  |
| San Mateo       | 137        | 0.0  | 7.30 | 0.0  | -     | -    |
| Santa Barbara   | 4          | 0.0  | 0.0  | 0.0  | -     | -    |
| Santa Clara     | 394        | 0.0  | 0.0  | 0.0  | -     | -    |
| Shasta          | 8,022      | 0.25 | 0.75 | 0.87 | -     | -    |
| Siskiyou        | 998        | 0.0  | 0.0  | 0.0  | -     | -    |
| Solano          | 1,931      | 0.0  | 0.52 | 0.0  | -     | -    |
| Stanislaus      | 11,223     | 0.45 | 0.36 | 1.53 | -     | -    |
| Tehama          | 6,023      | 0.33 | 0.66 | 0.33 | 13.56 | 3.15 |
| Tulare          | 46,425     | 1.59 | 0.67 | 2.28 | 1.56  | 2.17 |
| Ventura         | 860        | 0.0  | 0.0  | 0.0  | -     | -    |
| Yuba-Sutter     | 51,282     | 0.18 | 0.68 | 0.25 | 5.72  | 3.78 |
| Uncoded         | 22         | 0.0  | 0.0  | 0.0  | -     | -    |
| All             | 501,351    | 0.62 | 0.63 | 1.02 | 5.34  | 4.29 |

[a] MIR=Minimum infection rate/1000 *Cx. tarsalis*.

[b] Weighted mean monthly seroconversion (%) for the 5 year period.

Table IV-6. Yearly summaries in 6 California counties for light trap indices (LTI), viral isolations from female Cx. tarsalis and acquisition of antibody by rural sentinel chickens, 1978-1982.

| County<br>and<br>year | LTI<br>(rural) | No.<br><u>Cx.</u><br><u>tarsalis</u><br>tested | MIR [a] |      |      | Chicken [b]<br>Seroconversions |       |
|-----------------------|----------------|--|---------|------|------|--------------------------------|-------|
|                       |                |  | HP      | TUR  | WEE  | TUR                            | WEE   |
|                       |                |  |         |      |      |                                |       |
| <b>Butte</b>          |                |  |         |      |      |                                |       |
| 1978                  | 18.95          | 4,675  | 0.0     | 0.21 | 1.71 | 8.62                           | 7.47  |
| 1979                  | 15.33          | 5,225  | 0.0     | 0.0  | 0.19 | 10.96                          | 4.90  |
| 1980                  | 12.22          | 13,956   | 0.07    | 0.0  | 0.07 | 1.52                           | 0.37  |
| 1981                  | 19.82          | 8,236  | 0.85    | 0.48 | 0.24 | 8.25                           | 0.88  |
| 1982                  | 11.30          | 4,669  | 0.21    | 0.43 | 0.00 | 8.26                           | 0.0   |
| <b>Sutter-Yuba</b>    |                |  |         |      |      |                                |       |
| 1978                  | 5.01           | 690  | 0.0     | 1.45 | 0.0  | 5.10                           | 4.50  |
| 1979                  | 3.39           | 3,915  | 0.0     | 0.51 | 1.27 | 6.11                           | 8.01  |
| 1980                  | 1.53           | 24,269   | 0.12    | 0.21 | 0.16 | 3.78                           | 1.50  |
| 1981                  | 12.13          | 13,024   | 0.15    | 1.23 | 0.31 | 5.51                           | 0.0   |
| 1982                  | 9.63           | 9,384  | 0.43    | 1.17 | 0.0  | 16.00                          | 0.0   |
| <b>Tulare</b>         |                |  |         |      |      |                                |       |
| 1978                  | 0.81           | 5,234  | 1.91    | 0.0  | 0.57 | -                              | -     |
| 1979                  | 0.76           | 4,446  | 0.22    | 0.22 | 3.15 | 0.0                            | 0.0   |
| 1980                  | 0.22           | 9,324  | 1.72    | 0.54 | 0.54 | 0.0                            | 0.0   |
| 1981                  | 0.52           | 12,690   | 2.60    | 1.34 | 0.24 | 1.58                           | 0.78  |
| 1982                  | 0.43           | 14,727   | 0.95    | 0.54 | 5.50 | 2.59                           | 5.10  |
| <b>Kern</b>           |                |  |         |      |      |                                |       |
| 1978                  | 3.13           | 1,390  | 1.43    | 0.0  | 0.72 | 4.20                           | 2.54  |
| 1979                  | 3.97           | 32,651   | 0.12    | 0.52 | 1.10 | 7.51                           | 6.04  |
| 1980                  | 3.09           | 24,004   | 1.50    | 0.21 | 0.71 | 5.02                           | 5.25  |
| 1981                  | 2.05           | 29,959   | 1.56    | 0.53 | 0.63 | 8.21                           | 4.26  |
| 1982                  | 2.14           | 39,336   | 2.08    | 2.05 | 2.11 | 16.08                          | 17.20 |



Table IV-6. (cont'd)

| County           | LTI  | No.<br>tested | HP   | TUR  | WEE  | TUR  | WEE   |
|------------------|------|---------------|------|------|------|------|-------|
| <b>Imperial</b>  |      |               |      |      |      |      |       |
| 1978             | 0.60 | 5,256         | 0.0  | 0.76 | 1.52 | -    | -     |
| 1979             | 4.75 | 5,596         | 0.0  | 0.71 | 4.46 | 2.70 | 6.25  |
| 1980             | 8.49 | 8,878         | 0.0  | 0.22 | 0.79 | 3.37 | 17.18 |
| 1981             | 1.78 | 3,996         | 0.0  | 0.25 | 0.25 | 0.0  | 4.08  |
| 1982             | 1.86 | 6,675         | 0.0  | 0.45 | 4.49 | 4.72 | 31.53 |
| <b>Riverside</b> |      |               |      |      |      |      |       |
| 1978             | 0.97 | 21,220        | 0.05 | 0.38 | 1.65 | -    | -     |
| 1979             | 1.45 | 13,747        | 0.15 | 0.65 | 0.87 | 0.0  | 0.0   |
| 1980             | 1.25 | 12,791        | 0.08 | 0.31 | 1.09 | 1.02 | 1.05  |
| 1981             | 0.80 | 6,688         | 0.15 | 0.0  | 2.54 | 3.50 | 5.67  |
| 1982             | 4.17 | 12,071        | 0.25 | 0.58 | 0.58 | 0.0  | 4.55  |

[a] MIR=Minimum infection rate/1000 Cx. tarsalis.

[b] Weighted mean monthly seroconversion (%) for each yearly period.

Table IV-7. Number of Cx. tarsalis collected and tested for virus; light trap indices (LTI); infection rates for Hart Park, Turlock, western equine encephalomyelitis viruses per 1000 Cx. tarsalis; and percent of susceptible chickens infected, Butte County, 1978-1982.

| Year<br>and<br>month<br>collected | No.<br><u>Cx.</u><br><u>tarsalis</u><br>tested | LTI<br>(rural) | MIR [a] |      |      | Chicken [b]<br>seroconversions |       |
|-----------------------------------|--|----------------|---------|------|------|--------------------------------|-------|
|                                   |  |                | HP      | TUR  | WEE  | TUR                            | WEE   |
|                                   |  |                | -----   |      |      |                                |       |
| 1978                              |  |                |         |      |      |                                |       |
| January                           | -  | -              | -       | -    | -    | -                              | -     |
| February                          | -  | -              | -       | -    | -    | -                              | -     |
| March                             | -  | -              | -       | -    | -    | -                              | -     |
| April                             | -  | 0.62           | -       | -    | -    | -                              | -     |
| May                               | -  | 2.16           | -       | -    | -    | 0.0                            | 0.0   |
| June                              | 1,111  | 22.44          | 0.0     | 0.0  | 0.0  | 4.44                           | 0.0   |
| July                              | 950  | 55.23          | 0.0     | 1.05 | 0.0  | 6.98                           | 0.0   |
| August                            | 1,211  | 82.18          | 0.0     | 0.0  | 1.65 | 15.00                          | 24.44 |
| September                         | 1,000  | 32.11          | 0.0     | 0.0  | 6.00 | 26.47                          | 20.59 |
| October                           | 403  | 66.61          | 0.0     | 0.0  | 0.0  | 0.0                            | 0.0   |
| November                          | -  | -              | -       | -    | -    | -                              | -     |
| December                          | -  | -              | -       | -    | -    | -                              | -     |
| 1979                              |  |                |         |      |      |                                |       |
| January                           | -  | -              | -       | -    | -    | -                              | -     |
| February                          | -  | -              | -       | -    | -    | -                              | -     |
| March                             | -  | -              | -       | -    | -    | -                              | -     |
| April                             | -  | 0.58           | -       | -    | -    | -                              | -     |
| May                               | -  | 1.45           | -       | -    | -    | 0.0                            | 0.0   |
| June                              | 1,000  | 14.85          | 0.0     | 0.0  | 0.0  | 11.11                          | 0.0   |
| July                              | 2,675  | 46.86          | 0.0     | 0.0  | 0.37 | 7.50                           | 11.11 |
| August                            | 550  | 51.48          | 0.0     | 0.0  | 0.0  | 16.22                          | 7.50  |
| September                         | 500  | 39.74          | 0.0     | 0.0  | 0.0  | 32.26                          | 10.81 |
| October                           | 500  | 47.98          | 0.0     | 0.0  | 0.0  | -                              | -     |
| November                          | -  | -              | -       | -    | -    | -                              | -     |
| December                          | -  | -              | -       | -    | -    | -                              | -     |

Table IV-7. (cont'd)

| Month     | No.   | LTI   | HP   | TUR  | WEE  | TUR   | WEE  |
|-----------|-------|-------|------|------|------|-------|------|
| 1980      |       |       |      |      |      |       |      |
| January   | -     | -     | -    | -    | -    | -     | -    |
| February  | -     | -     | -    | -    | -    | -     | -    |
| March     | -     | -     | -    | -    | -    | -     | -    |
| April     | -     | 0.32  | -    | -    | -    | -     | -    |
| May       | 13    | 1.09  | 0.0  | 0.0  | 0.0  | 0.0   | 0.0  |
| June      | 1,406 | 14.85 | 0.0  | 0.0  | 0.0  | 0.0   | 0.0  |
| July      | 6,329 | 33.67 | 0.16 | 0.0  | 0.0  | 2.22  | 0.0  |
| August    | 4,309 | 47.98 | 0.0  | 0.0  | 0.0  | 0.0   | 2.22 |
| September | 1,899 | 35.31 | 0.0  | 0.0  | 0.53 | 6.82  | 0.0  |
| October   | -     | 25.30 | -    | -    | -    | -     | -    |
| November  | -     | -     | -    | -    | -    | -     | -    |
| December  | -     | -     | -    | -    | -    | -     | -    |
| 1981      |       |       |      |      |      |       |      |
| January   | -     | -     | -    | -    | -    | -     | -    |
| February  | -     | -     | -    | -    | -    | -     | -    |
| March     | -     | -     | -    | -    | -    | -     | -    |
| April     | -     | 0.99  | -    | -    | -    | -     | -    |
| May       | -     | 2.24  | -    | -    | -    | 0.0   | 0.0  |
| June      | 950   | 23.55 | 3.16 | 0.0  | 0.0  | 0.0   | 0.0  |
| July      | 2,814 | 55.23 | 1.07 | 1.42 | 0.36 | 10.53 | 0.0  |
| August    | 2,751 | 51.48 | 0.36 | 0.0  | 0.0  | 29.41 | 0.0  |
| September | 1,721 | 96.72 | 0.0  | 0.0  | 0.58 | 8.33  | 5.26 |
| October   | -     | 36.15 | -    | -    | -    | -     | -    |
| November  | -     | -     | -    | -    | -    | -     | -    |
| December  | -     | -     | -    | -    | -    | -     | -    |
| 1982      |       |       |      |      |      |       |      |
| January   | -     | -     | -    | -    | -    | -     | -    |
| February  | -     | -     | -    | -    | -    | -     | -    |
| March     | -     | -     | -    | -    | -    | -     | -    |
| April     | -     | 0.38  | -    | -    | -    | -     | -    |
| May       | 44    | 0.55  | 0.0  | 0.0  | 0.0  | 0.0   | 0.0  |
| June      | 500   | 5.45  | 0.0  | 0.0  | 0.0  | 0.0   | 0.0  |
| July      | 1,250 | 35.31 | 0.0  | 0.0  | 0.0  | 4.76  | 0.0  |
| August    | 2,125 | 39.74 | 0.0  | 0.0  | 0.0  | 30.00 | 0.0  |
| September | 750   | 33.67 | 1.33 | 0.0  | 0.0  | 14.28 | 0.0  |
| October   | -     | 59.26 | -    | -    | -    | -     | -    |
| November  | -     | -     | -    | -    | -    | -     | -    |
| December  | -     | -     | -    | -    | -    | -     | -    |

[a] MIR=Minimum infection rate/1000 Cx. tarsalis.

[b] Monthly seroconversion (%).

Table IV-8. Number of *Cx. tarsalis* collected and tested for virus; light trap indices (LTI); infection rates for Hart Park, Turlock, western equine encephalomyelitis viruses per 1000 *Cx. tarsalis*; and percent of susceptible chickens infected, Imperial County, 1978-1982.

| Year<br>and<br>month<br>collected | No.<br><i>Cx.</i><br><i>tarsalis</i> | LTI<br>(rural) | MIR [a] |      |      | Chicken [b]<br>seroconversions |       |
|-----------------------------------|--------------------------------------|----------------|---------|------|------|--------------------------------|-------|
|                                   |                                      |                | HP      | TUR  | WEE  | TUR                            | WEE   |
|                                   |                                      |                | -----   |      |      |                                |       |
| 1978                              |                                      |                |         |      |      |                                |       |
| January                           | -                                    | -              | -       | -    | -    | -                              | -     |
| February                          | -                                    | -              | -       | -    | -    | -                              | -     |
| March                             | -                                    | -              | -       | -    | -    | -                              | -     |
| April                             | 769                                  | 1.13           | 0.0     | 1.30 | 0.0  | -                              | -     |
| May                               | 1 092                                | 0.78           | 0.0     | 0.92 | 0.0  | -                              | -     |
| June                              | 2,678                                | 0.45           | 0.0     | 0.75 | 2.61 | -                              | -     |
| July                              | 242                                  | 0.38           | 0.0     | 0.0  | 0.0  | -                              | -     |
| August                            | 361                                  | 0.91           | 0.0     | 0.0  | 2.77 | -                              | -     |
| September                         | 114                                  | 0.20           | 0.0     | 0.0  | 0.00 | -                              | -     |
| October                           | -                                    | 0.51           | -       | -    | -    | -                              | -     |
| November                          | -                                    | -              | -       | -    | -    | -                              | -     |
| December                          | -                                    | -              | -       | -    | -    | -                              | -     |
| 1979                              |                                      |                |         |      |      |                                |       |
| January                           | -                                    | -              | -       | -    | -    | -                              | -     |
| February                          | -                                    | -              | -       | -    | -    | -                              | -     |
| March                             | -                                    | -              | -       | -    | -    | -                              | -     |
| April                             | 464                                  | 8.77           | 0.0     | 0.0  | 0.0  | -                              | -     |
| May                               | -                                    | 10.22          | -       | -    | -    | 0.0                            | 0.0   |
| June                              | 2,638                                | 3.17           | 0.0     | 1.14 | 7.96 | 0.0                            | 0.0   |
| July                              | 671                                  | 5.17           | 0.0     | 0.0  | 0.0  | 0.0                            | 31.58 |
| August                            | 1,224                                | 3.17           | 0.0     | 0.82 | 3.27 | 0.0                            | 0.0   |
| September                         | 599                                  | 4.13           | 0.0     | 0.0  | 0.0  | 15.79                          | 0.0   |
| October                           | -                                    | 2.47           | -       | -    | -    | -                              | -     |
| November                          | -                                    | -              | -       | -    | -    | -                              | -     |
| December                          | -                                    | -              | -       | -    | -    | -                              | -     |

Table IV-8. (cont'd)

| Month     | No.   | LTI   | HP  | TUR  | WEE  | TUR   | WEE   |
|-----------|-------|-------|-----|------|------|-------|-------|
| 1980      |       |       |     |      |      |       |       |
| January   | -     | -     | -   | -    | -    | -     | -     |
| February  | 46    | -     | 0.0 | 0.0  | 0.0  | -     | -     |
| March     | 268   | -     | 0.0 | 0.0  | 0.0  | -     | -     |
| April     | 1,401 | 5.92  | 0.0 | 0.0  | 0.0  | -     | -     |
| May       | 3,240 | 11.88 | 0.0 | 0.0  | 0.0  | 0.0   | 0.0   |
| June      | 1,485 | 17.62 | 0.0 | 0.0  | 0.0  | 0.0   | 18.75 |
| July      | 936   | 16.37 | 0.0 | 2.14 | 4.27 | 6.25  | 46.15 |
| August    | 257   | 8.33  | 0.0 | 0.0  | 0.0  | 13.33 | 0.0   |
| September | 1,084 | 3.79  | 0.0 | 0.0  | 2.77 | 0.0   | 28.57 |
| October   | 122   | 4.73  | 0.0 | 0.0  | 0.0  | -     | -     |
| November  | 37    | -     | 0.0 | 0.0  | 0.0  | -     | -     |
| December  | -     | -     | -   | -    | -    | -     | -     |
| 1981      |       |       |     |      |      |       |       |
| January   | -     | -     | -   | -    | -    | -     | -     |
| February  | -     | -     | -   | -    | -    | -     | -     |
| March     | 449   | -     | 0.0 | 0.0  | 0.0  | -     | -     |
| April     | 2,545 | 0.86  | 0.0 | 0.0  | 0.0  | -     | -     |
| May       | -     | 5.31  | -   | -    | -    | 0.0   | 0.0   |
| June      | 864   | 3.79  | 0.0 | 1.16 | 1.16 | 0.0   | 0.0   |
| July      | 138   | 1.09  | 0.0 | 0.0  | 0.0  | 0.0   | 16.67 |
| August    | -     | 3.37  | -   | -    | -    | 0.0   | 0.0   |
| September | -     | 0.95  | -   | -    | -    | 0.0   | 6.67  |
| October   | -     | 0.29  | -   | -    | -    | -     | -     |
| November  | -     | -     | -   | -    | -    | -     | -     |
| December  | -     | -     | -   | -    | -    | -     | -     |
| 1982      |       |       |     |      |      |       |       |
| January   | -     | -     | -   | -    | -    | -     | -     |
| February  | -     | -     | -   | -    | -    | -     | -     |
| March     | -     | -     | -   | -    | -    | -     | -     |
| April     | -     | 3.79  | -   | -    | -    | -     | -     |
| May       | 2,824 | 4.75  | 0.0 | 0.35 | 0.71 | 0.0   | 5.13  |
| June      | 3,099 | 5.76  | 0.0 | 0.32 | 9.04 | 0.0   | 45.95 |
| July      | -     | 1.04  | -   | -    | -    | 10.25 | 65.00 |
| August    | 329   | 0.66  | 0.0 | 0.0  | 0.0  | 11.43 | 42.86 |
| September | 384   | 0.78  | 0.0 | 2.60 | 0.0  | 6.45  | 0.0   |
| October   | 39    | -     | 0.0 | 0.0  | 0.0  | -     | -     |
| November  | -     | -     | -   | -    | -    | -     | -     |
| December  | -     | -     | -   | -    | -    | -     | -     |

[a] MIR=Minimum infection rate/1000 Cx. tarsalis.

[b] Monthly seroconversion (%).

Table IV-9. Number of Cx. tarsalis collected and tested for virus; light trap indices (LTI); infection rates for Hart Park, Turlock, western equine encephalomyelitis viruses per 1000 Cx. tarsalis; and percent of susceptible chickens infected, Kern County, 1978-1982.

| Year<br>and<br>month<br>collected | No.<br><u>Cx.</u><br><u>tarsalis</u> | LTI<br>(rural) | MIR [a] |      |      | Chicken [b]<br>seroconversions |       |
|-----------------------------------|--------------------------------------|----------------|---------|------|------|--------------------------------|-------|
|                                   |                                      |                | HP      | TUR  | WEE  | TUR                            | WEE   |
|                                   |                                      |                | -----   |      |      |                                |       |
| 1978                              |                                      |                |         |      |      |                                |       |
| January                           | 99                                   | -              | 0.0     | 0.0  | 0.0  | -                              | -     |
| February                          | -                                    | -              | -       | -    | -    | -                              | -     |
| March                             | -                                    | -              | -       | -    | -    | -                              | -     |
| April                             | -                                    | 1.42           | -       | -    | -    | -                              | -     |
| May                               | 31                                   | 3.54           | 0.0     | 0.0  | 0.0  | 0.55                           | 0.0   |
| June                              | 638                                  | 10.75          | 3.13    | 0.0  | 0.0  | 0.55                           | 0.0   |
| July                              | 76                                   | 3.71           | 0.0     | 0.0  | 0.0  | 3.89                           | 2.20  |
| August                            | -                                    | 1.34           | -       | -    | -    | 6.94                           | 2.81  |
| September                         | 170                                  | 1.97           | 0.0     | 0.0  | 5.88 | 8.70                           | 4.05  |
| October                           | 376                                  | 3.86           | 0.0     | 0.0  | 0.0  | 5.44                           | 6.63  |
| November                          | -                                    | -              | -       | -    | -    | -                              | -     |
| December                          | -                                    | -              | -       | -    | -    | -                              | -     |
| 1979                              |                                      |                |         |      |      |                                |       |
| January                           | -                                    | -              | -       | -    | -    | -                              | -     |
| February                          | -                                    | -              | -       | -    | -    | -                              | -     |
| March                             | -                                    | -              | -       | -    | -    | -                              | -     |
| April                             | -                                    | 0.32           | -       | -    | -    | -                              | -     |
| May                               | 3,462                                | 1.40           | 0.0     | 0.29 | 0.0  | 0.0                            | 0.0   |
| June                              | 5,103                                | 2.95           | 0.78    | 1.18 | 0.0  | 10.90                          | 0.0   |
| July                              | 6,031                                | 4.25           | 0.0     | 0.83 | 0.33 | 2.16                           | 12.82 |
| August                            | 8,031                                | 10.13          | 0.0     | 0.25 | 1.74 | 12.50                          | 5.15  |
| September                         | 8,273                                | 15.34          | 0.0     | 0.36 | 2.05 | 10.92                          | 22.48 |
| October                           | 1,751                                | 5.30           | 0.0     | 0.0  | 1.71 | 10.38                          | 1.00  |
| November                          | -                                    | -              | -       | -    | -    | -                              | -     |
| December                          | -                                    | -              | -       | -    | -    | -                              | -     |

Table IV-9. (cont'd)

| Month     | No.   | LTI  | HP   | TUR  | WEE  | TUR   | WEE   |
|-----------|-------|------|------|------|------|-------|-------|
| 1980      |       |      |      |      |      |       |       |
| January   | -     | -    | -    | -    | -    | -     | -     |
| February  | -     | -    | -    | -    | -    | -     | -     |
| March     | -     | -    | -    | -    | -    | -     | -     |
| April     | 450   | 0.32 | 0.0  | 0.0  | 0.0  | -     | -     |
| May       | 1,783 | 1.71 | 0.0  | 0.0  | 0.0  | 0.52  | 0.0   |
| June      | 4,527 | 2.36 | 2.11 | 0.23 | 0.94 | 0.52  | 0.0   |
| July      | 6,185 | 4.58 | 3.23 | 0.16 | 0.0  | 1.06  | 0.0   |
| August    | 6,167 | 5.97 | 0.97 | 0.49 | 1.30 | 14.44 | 12.04 |
| September | 3,800 | 7.19 | 0.26 | 0.0  | 1.32 | 13.75 | 19.64 |
| October   | 1,362 | 3.97 | 0.0  | 0.0  | 0.0  | -     | -     |
| November  | -     | -    | -    | -    | -    | -     | -     |
| December  | -     | -    | -    | -    | -    | -     | -     |
| 1981      |       |      |      |      |      |       |       |
| January   | -     | -    | -    | -    | -    | -     | -     |
| February  | -     | -    | -    | -    | -    | -     | -     |
| March     | -     | -    | -    | -    | -    | -     | -     |
| April     | -     | 0.58 | -    | -    | -    | -     | -     |
| May       | 1,642 | 1.80 | 0.0  | 0.0  | 0.0  | 0.0   | 0.0   |
| June      | 3,168 | 1.44 | 2.21 | 0.0  | 0.0  | 0.0   | 0.0   |
| July      | 7,259 | 2.09 | 3.30 | 0.41 | 0.0  | 4.40  | 0.0   |
| August    | 7,943 | 3.20 | 1.64 | 0.63 | 0.76 | 27.59 | 8.79  |
| September | 9,947 | 4.37 | 0.30 | 0.80 | 1.31 | 17.46 | 16.87 |
| October   | -     | 2.26 | -    | -    | -    | -     | -     |
| November  | -     | -    | -    | -    | -    | -     | -     |
| December  | -     | -    | -    | -    | -    | -     | -     |
| 1982      |       |      |      |      |      |       |       |
| January   | -     | -    | -    | -    | -    | -     | -     |
| February  | -     | -    | -    | -    | -    | -     | -     |
| March     | -     | -    | -    | -    | -    | -     | -     |
| April     | -     | 0.45 | -    | -    | -    | -     | -     |
| May       | 3,669 | 1.67 | 0.54 | 1.09 | 0.0  | 0.0   | 0.0   |
| June      | 8,855 | 3.17 | 3.39 | 3.39 | 0.0  | 0.0   | 0.0   |
| July      | 7,997 | 2.60 | 4.62 | 3.50 | 0.25 | 15.00 | 1.25  |
| August    | 9,632 | 3.30 | 1.14 | 0.93 | 4.36 | 44.12 | 53.16 |
| September | 8,633 | 4.25 | 0.12 | 0.93 | 4.40 | 44.74 | 56.76 |
| October   | -     | 1.33 | -    | -    | -    | -     | -     |
| November  | -     | -    | -    | -    | -    | -     | -     |
| December  | -     | -    | -    | -    | -    | -     | -     |

(a) MIR=Minimum infection rate/1000 *Cx. tarsalis*.

(b) Monthly seroconversion (%).

Table IV-10. Number of Cx. tarsalis collected and tested for virus; light trap indices (LTI); infection rates for Hart Park, Turlock, western equine encephalomyelitis viruses per 1000 Cx. tarsalis; and percent of susceptible chickens infected, Riverside County, 1978-1982.

| Year<br>and<br>month<br>collected | No.<br><u>Cx.</u><br><u>tarsalis</u> | LTI<br>(rural) | MIR [a] |      |      | Chicken [b]<br>seroconversions |     |
|-----------------------------------|--------------------------------------|----------------|---------|------|------|--------------------------------|-----|
|                                   |                                      |                | HP      | TUR  | WEE  | TUR                            | WEE |
|                                   |                                      |                |         |      |      |                                |     |
| -----                             |                                      |                |         |      |      |                                |     |
| 1978                              |                                      |                |         |      |      |                                |     |
| January                           | -                                    | -              | -       | -    | -    | -                              | -   |
| February                          | -                                    | -              | -       | -    | -    | -                              | -   |
| March                             | -                                    | -              | -       | -    | -    | -                              | -   |
| April                             | 2,383                                | 1.04           | 0.42    | 0.0  | 0.0  | -                              | -   |
| May                               | 3,989                                | 2.24           | 0.0     | 1.25 | 0.50 | -                              | -   |
| June                              | 3,646                                | 1.30           | 0.0     | 0.0  | 6.58 | -                              | -   |
| July                              | 2,978                                | 0.84           | 0.0     | 0.34 | 2.69 | -                              | -   |
| August                            | 2,218                                | 0.60           | 0.0     | 0.0  | 0.45 | -                              | -   |
| September                         | 5,520                                | 0.44           | 0.0     | 0.36 | 0.0  | -                              | -   |
| October                           | 486                                  | 0.82           | 0.0     | 0.0  | 0.0  | -                              | -   |
| November                          | -                                    | -              | -       | -    | -    | -                              | -   |
| December                          | -                                    | -              | -       | -    | -    | -                              | -   |
| -----                             |                                      |                |         |      |      |                                |     |
| 1979                              |                                      |                |         |      |      |                                |     |
| January                           | -                                    | -              | -       | -    | -    | -                              | -   |
| February                          | -                                    | -              | -       | -    | -    | -                              | -   |
| March                             | -                                    | -              | -       | -    | -    | -                              | -   |
| April                             | 950                                  | 1.60           | 0.0     | 0.0  | 0.0  | -                              | -   |
| May                               | 3,023                                | 2.85           | 0.33    | 1.32 | 0.0  | 0.0                            | 0.0 |
| June                              | 3,219                                | 2.76           | 0.0     | 0.31 | 1.86 | 0.0                            | 0.0 |
| July                              | 1,832                                | 1.00           | 0.0     | 1.09 | 1.63 | 0.0                            | 0.0 |
| August                            | 2,069                                | 0.80           | 0.48    | 0.97 | 1.45 | 0.0                            | 0.0 |
| September                         | 1,608                                | 0.75           | 0.0     | 0.0  | 0.0  | 0.0                            | 0.0 |
| October                           | 1,046                                | 1.21           | 0.0     | 0.0  | 0.0  | -                              | -   |
| November                          | -                                    | -              | -       | -    | -    | -                              | -   |
| December                          | -                                    | -              | -       | -    | -    | -                              | -   |
| -----                             |                                      |                |         |      |      |                                |     |



Table IV-10. (cont'd)

| Month     | No.   | LTI  | HP   | TUR  | WEE   | TUR   | WEE   |
|-----------|-------|------|------|------|-------|-------|-------|
| 1980      |       |      |      |      |       |       |       |
| January   | -     | -    | -    | -    | -     | -     | -     |
| February  | -     | -    | -    | -    | -     | -     | -     |
| March     | -     | -    | -    | -    | -     | -     | -     |
| April     | 1,613 | 1.24 | 0.0  | 0.0  | 0.0   | -     | -     |
| May       | 2,498 | 2.55 | 0.0  | 0.0  | 0.0   | 0.0   | 0.0   |
| June      | 2,692 | 2.31 | 0.0  | 0.37 | 1.86  | 3.03  | 0.0   |
| July      | 3,447 | 1.63 | 0.29 | 0.87 | 1.74  | 3.12  | 0.0   |
| August    | 2,022 | 0.60 | 0.0  | 0.0  | 1.48  | 0.0   | 0.0   |
| September | 519   | 0.51 | 0.0  | 0.0  | 0.0   | 0.0   | 0.0   |
| October   | -     | 0.71 | -    | -    | -     | -     | -     |
| November  | -     | -    | -    | -    | -     | -     | -     |
| December  | -     | -    | -    | -    | -     | -     | -     |
| 1981      |       |      |      |      |       |       |       |
| January   | -     | -    | -    | -    | -     | -     | -     |
| February  | -     | -    | -    | -    | -     | -     | -     |
| March     | -     | -    | -    | -    | -     | -     | -     |
| April     | 477   | 0.93 | 0.0  | 0.0  | 0.0   | -     | -     |
| May       | 1,600 | 0.92 | 0.63 | 0.0  | 1.25  | 2.70  | 0.0   |
| June      | 900   | 1.07 | 0.0  | 0.0  | 13.33 | 0.0   | 5.41  |
| July      | 942   | 0.65 | 0.0  | 0.0  | 3.18  | 11.11 | 11.43 |
| August    | 1,408 | 0.57 | 0.0  | 0.0  | 0.0   | 15.63 | 3.23  |
| September | 1,361 | 0.65 | 0.0  | 0.0  | 0.0   | 3.70  | 0.0   |
| October   | -     | 0.75 | -    | -    | -     | -     | -     |
| November  | -     | -    | -    | -    | -     | -     | -     |
| December  | -     | -    | -    | -    | -     | -     | -     |
| 1982      |       |      |      |      |       |       |       |
| January   | -     | -    | -    | -    | -     | -     | -     |
| February  | -     | -    | -    | -    | -     | -     | -     |
| March     | -     | -    | -    | -    | -     | -     | -     |
| April     | 1,136 | 1.93 | 0.0  | 0.88 | 0.0   | -     | -     |
| May       | 1,250 | 4.16 | 1.60 | 1.60 | 0.0   | 0.0   | 0.0   |
| June      | 1,461 | 8.33 | 0.0  | 0.0  | 0.0   | 6.06  | 0.0   |
| July      | 2,180 | 8.54 | 0.46 | 0.46 | 0.46  | 6.45  | 0.0   |
| August    | 2,144 | 6.13 | 0.0  | 1.40 | 2.80  | 13.79 | 0.0   |
| September | 3,400 | 2.38 | 0.0  | 0.0  | 0.0   | 0.0   | 0.0   |
| October   | 500   | 2.01 | 0.0  | 0.0  | 0.0   | 0.0   | 0.0   |
| November  | -     | -    | -    | -    | -     | -     | -     |
| December  | -     | -    | -    | -    | -     | -     | -     |

(a) MIR=Minimum infection rate/1000 Cx. tarsalis.

(b) Monthly seroconversion (%).

Table IV-11. Number of Cx. tarsalis collected and tested for virus; light trap indices (LTI); infection rates for Hart Park, Turlock, western equine encephalomyelitis viruses per 1000 Cx. tarsalis; and percent of susceptible chickens infected, Tulare County, 1978-1982.

| Year<br>and<br>county<br>collected | No.<br><u>Cx.</u><br><u>tarsalis</u> | LTI<br>(rural) | MIR [a] |      |      | Chicken [b]<br>seroconversions |     |
|------------------------------------|--------------------------------------|----------------|---------|------|------|--------------------------------|-----|
|                                    |                                      |                | HP      | TUR  | WEE  | TUR                            | WEE |
|                                    |                                      |                | -----   |      |      |                                |     |
| 1978                               |                                      |                |         |      |      |                                |     |
| January                            | -                                    | -              | -       | -    | -    | -                              | -   |
| February                           | -                                    | -              | -       | -    | -    | -                              | -   |
| March                              | -                                    | -              | -       | -    | -    | -                              | -   |
| April                              | -                                    | 0.40           | -       | -    | -    | -                              | -   |
| May                                | 401                                  | 2.24           | 12.46   | 0.0  | 0.0  | -                              | -   |
| June                               | 486                                  | 1.32           | 10.28   | 0.0  | 0.0  | -                              | -   |
| July                               | -                                    | 0.60           | -       | -    | -    | -                              | -   |
| August                             | 4,079                                | 0.43           | 0.0     | 0.0  | 0.49 | -                              | -   |
| September                          | 52                                   | 0.58           | 0.0     | 0.0  | 0.0  | -                              | -   |
| October                            | 220                                  | 0.51           | 0.0     | 0.0  | 4.55 | -                              | -   |
| November                           | -                                    | -              | -       | -    | -    | -                              | -   |
| December                           | -                                    | -              | -       | -    | -    | -                              | -   |
| 1979                               |                                      |                |         |      |      |                                |     |
| January                            | -                                    | -              | -       | -    | -    | -                              | -   |
| February                           | -                                    | -              | -       | -    | -    | -                              | -   |
| March                              | -                                    | -              | -       | -    | -    | -                              | -   |
| April                              | -                                    | -              | -       | -    | -    | -                              | -   |
| May                                | 39                                   | 0.19           | 0.0     | 0.0  | 0.0  | 0.0                            | 0.0 |
| June                               | 127                                  | 0.41           | 0.0     | 0.0  | 0.0  | 0.0                            | 0.0 |
| July                               | 350                                  | 0.39           | 2.86    | 0.0  | 0.0  | 0.0                            | 0.0 |
| August                             | -                                    | 2.24           | -       | -    | -    | 0.0                            | 0.0 |
| September                          | 2,795                                | 3.27           | 0.0     | 0.36 | 4.65 | 0.0                            | 0.0 |
| October                            | 1,135                                | 0.60           | 0.0     | 0.0  | 0.88 | 0.0                            | 0.0 |
| November                           | -                                    | -              | -       | -    | -    | -                              | -   |
| December                           | -                                    | -              | -       | -    | -    | -                              | -   |

Table IV-11 (cont'd)

| Month     | No.   | LTI  | HP    | TUR  | WEE  | TUR   | WEE   |
|-----------|-------|------|-------|------|------|-------|-------|
| 1980      |       |      |       |      |      |       |       |
| January   | -     | -    | -     | -    | -    | -     | -     |
| February  | -     | -    | -     | -    | -    | -     | -     |
| March     | -     | -    | -     | -    | -    | -     | -     |
| April     | -     | 0.01 | -     | -    | -    | -     | -     |
| May       | -     | 0.17 | -     | -    | -    | 0.0   | 0.0   |
| June      | 64    | 0.21 | 15.62 | 0.0  | 0.0  | 0.0   | 0.0   |
| July      | 911   | 0.45 | 5.49  | 0.0  | 0.0  | 0.0   | 0.0   |
| August    | 5,443 | 0.40 | 1.65  | 0.73 | 0.55 | 0.0   | 0.0   |
| September | 2,906 | 0.26 | 0.34  | 0.34 | 0.68 | 0.0   | 0.0   |
| October   | -     | 0.11 | -     | -    | -    | -     | -     |
| November  | -     | -    | -     | -    | -    | -     | -     |
| December  | -     | -    | -     | -    | -    | -     | -     |
| 1981      |       |      |       |      |      |       |       |
| January   | -     | -    | -     | -    | -    | -     | -     |
| February  | -     | -    | -     | -    | -    | -     | -     |
| March     | -     | -    | -     | -    | -    | -     | -     |
| April     | -     | 0.04 | -     | -    | -    | -     | -     |
| May       | -     | 0.64 | -     | -    | -    | 0.0   | 0.0   |
| June      | 17    | 0.36 | 0.0   | 0.0  | 0.0  | 0.0   | 0.0   |
| July      | 1,169 | 0.16 | 7.70  | 0.86 | 0.0  | 0.0   | 0.0   |
| August    | 9,517 | 1.00 | 2.52  | 1.57 | 0.0  | 2.33  | 0.0   |
| September | 1,987 | 1.24 | 0.0   | 0.50 | 1.51 | 7.14  | 4.65  |
| October   | -     | 0.55 | -     | -    | -    | -     | -     |
| November  | -     | -    | -     | -    | -    | -     | -     |
| December  | -     | -    | -     | -    | -    | -     | -     |
| 1982      |       |      |       |      |      |       |       |
| January   | -     | -    | -     | -    | -    | -     | -     |
| February  | -     | -    | -     | -    | -    | -     | -     |
| March     | -     | -    | -     | -    | -    | -     | -     |
| April     | -     | 0.01 | -     | -    | -    | -     | -     |
| May       | 319   | 0.93 | 0.0   | 0.0  | 0.0  | 0.0   | 0.0   |
| June      | 515   | 0.71 | 7.76  | 0.0  | 0.0  | 0.0   | 0.0   |
| July      | 887   | 0.43 | 3.38  | 0.0  | 9.02 | 0.0   | 2.13  |
| August    | 9,547 | 0.62 | 0.73  | 0.73 | 6.08 | 10.64 | 26.09 |
| September | 3,549 | 0.50 | 0.0   | 0.29 | 4.33 | 4.76  | 0.0   |
| October   | -     | 0.05 | -     | -    | -    | -     | -     |
| November  | -     | -    | -     | -    | -    | -     | -     |
| December  | -     | -    | -     | -    | -    | -     | -     |

[a] MIR=Minimum infection rate/1000 Cx. tarsalis.

[b] Monthly seroconversion (%).

Table IV-12. Number of Cx. tarsalis collected and tested for virus; light trap indices (LTI); infection rates for Hart Park, Turlock, western equine encephalomyelitis viruses per 1000 Cx. tarsalis; and percent of susceptible chickens infected, Sutter-Yuba County, 1978-1982.

| Year<br>and<br>month<br>collected | No.<br><u>Cx.</u><br><u>tarsalis</u> | LTI<br>(rural) | MIR [a] |      |      | Chicken [b]<br>seroconversions |       |
|-----------------------------------|--------------------------------------|----------------|---------|------|------|--------------------------------|-------|
|                                   |                                      |                | HP      | TUR  | WEE  | TUR                            | WEE   |
|                                   |                                      |                |         |      |      |                                |       |
| -----                             |                                      |                |         |      |      |                                |       |
| 1978                              |                                      |                |         |      |      |                                |       |
| January                           | -                                    | -              | -       | -    | -    | -                              | -     |
| February                          | -                                    | -              | -       | -    | -    | -                              | -     |
| March                             | -                                    | -              | -       | -    | -    | -                              | -     |
| April                             | -                                    | 0.34           | -       | -    | -    | -                              | -     |
| May                               | 32                                   | 0.65           | 0.0     | 0.0  | 0.0  | 0.0                            | 1.12  |
| June                              | -                                    | 4.28           | -       | -    | -    | 5.62                           | 0.0   |
| July                              | 461                                  | 32.88          | 0.0     | 2.17 | 0.0  | 8.33                           | 10.23 |
| August                            | 142                                  | 24.70          | 0.0     | 0.0  | 0.0  | 12.99                          | 5.06  |
| September                         | 55                                   | 5.31           | 0.0     | 0.0  | 0.0  | 2.99                           | 10.67 |
| October                           | -                                    | 3.37           | -       | -    | -    | -                              | -     |
| November                          | -                                    | -              | -       | -    | -    | -                              | -     |
| December                          | -                                    | -              | -       | -    | -    | -                              | -     |
| -----                             |                                      |                |         |      |      |                                |       |
| 1979                              |                                      |                |         |      |      |                                |       |
| January                           | -                                    | -              | -       | -    | -    | -                              | -     |
| February                          | -                                    | -              | -       | -    | -    | -                              | -     |
| March                             | -                                    | -              | -       | -    | -    | -                              | -     |
| April                             | -                                    | 0.20           | -       | -    | -    | -                              | -     |
| May                               | -                                    | 0.28           | -       | -    | -    | 1.18                           | 0.0   |
| June                              | 391                                  | 3.79           | 0.0     | 0.0  | 0.0  | 5.95                           | 4.71  |
| July                              | 1,089                                | 10.22          | 0.0     | 1.84 | 3.67 | 10.13                          | 29.63 |
| August                            | 1,698                                | 22.98          | 0.0     | 0.0  | 0.59 | 8.45                           | 8.77  |
| September                         | 663                                  | 5.92           | 0.0     | 0.0  | 0.0  | 10.77                          | 0.0   |
| October                           | 74                                   | 1.29           | 0.0     | 0.0  | 0.0  | -                              | -     |
| November                          | -                                    | -              | -       | -    | -    | -                              | -     |
| December                          | -                                    | -              | -       | -    | -    | -                              | -     |

Table IV-12. (cont'd)

| Month     | No.   | LTI   | HP   | TUR  | WEE  | TUR   | WEE  |
|-----------|-------|-------|------|------|------|-------|------|
| 1980      |       |       |      |      |      |       |      |
| January   | -     | -     | -    | -    | -    | -     | -    |
| February  | -     | -     | -    | -    | -    | -     | -    |
| March     | -     | -     | -    | -    | -    | -     | -    |
| April     | -     | 0.02  | -    | -    | -    | -     | -    |
| May       | -     | 0.05  | -    | -    | -    | 0.0   | 0.0  |
| June      | 3,083 | 1.40  | 0.32 | 0.0  | 0.0  | 0.0   | 0.0  |
| July      | 9,166 | 9.47  | 0.22 | 0.0  | 0.0  | 1.25  | 1.25 |
| August    | 9,684 | 5.92  | 0.0  | 0.31 | 0.41 | 13.92 | 3.80 |
| September | 2,336 | 1.81  | 0.0  | 0.86 | 0.0  | 7.35  | 3.95 |
| October   | -     | 0.29  | -    | -    | -    | -     | -    |
| November  | -     | -     | -    | -    | -    | -     | -    |
| December  | -     | -     | -    | -    | -    | -     | -    |
| 1981      |       |       |      |      |      |       |      |
| January   | -     | -     | -    | -    | -    | -     | -    |
| February  | -     | -     | -    | -    | -    | -     | -    |
| March     | 100   | -     | 0.0  | 10.0 | 0.0  | -     | -    |
| April     | -     | 0.00  | -    | -    | -    | -     | -    |
| May       | -     | 2.31  | -    | -    | -    | 0.0   | 0.0  |
| June      | 2,575 | 20.88 | 0.78 | 2.33 | 0.0  | 0.0   | 0.0  |
| July      | 4,394 | 66.61 | 0.0  | 1.59 | 0.0  | 8.70  | 0.0  |
| August    | 5,005 | 44.71 | 0.0  | 0.40 | 0.40 | 0.0   | 0.0  |
| September | 950   | 21.91 | 0.0  | 0.0  | 2.11 | 23.81 | 0.0  |
| October   | -     | -     | -    | -    | -    | -     | -    |
| November  | -     | -     | -    | -    | -    | -     | -    |
| December  | -     | -     | -    | -    | -    | -     | -    |
| 1982      |       |       |      |      |      |       |      |
| January   | -     | -     | -    | -    | -    | -     | -    |
| February  | -     | -     | -    | -    | -    | -     | -    |
| March     | -     | -     | -    | -    | -    | -     | -    |
| April     | -     | 0.32  | -    | -    | -    | -     | -    |
| May       | -     | 0.78  | -    | -    | -    | 0.0   | 0.0  |
| June      | 926   | 7.71  | 0.0  | 0.0  | 0.0  | 4.35  | 0.0  |
| July      | 3,827 | 63.57 | 0.26 | 1.56 | 0.0  | 31.82 | 0.0  |
| August    | 4,066 | 53.95 | 0.74 | 1.22 | 0.0  | 33.33 | 0.0  |
| September | 565   | 18.95 | 0.0  | 0.0  | 0.0  | 30.00 | 0.0  |
| October   | -     | -     | -    | -    | -    | -     | -    |
| November  | -     | -     | -    | -    | -    | -     | -    |
| December  | -     | -     | -    | -    | -    | -     | -    |

[a] MIR=Minimum infection rate/1000 *Cx. tarsalis*.

[b] Monthly seroconversion (%).

Table IV-13. Correlation (r) (Pearson product moment) of western equine encephalomyelitis, Turlock, and Hart Park *Cx. tarsalis* minimum infection rates (MIR) with the rural light trap indices (LTI) of California counties, 1978-1982.

| County       | Mean             |              |        |       | r            |       |       |
|--------------|------------------|--------------|--------|-------|--------------|-------|-------|
|              | LTI              | WEEMIR       | TURMIR | HPMIR | WEE          | TUR   | HP    |
| Alameda      | 0.41<br>(35) [a] | 0.0<br>(3)   | 0.0    | 9.80  | 0.0<br>(3)   | 0.0   | 0.72  |
| Butte        | 14.85<br>(35)    | 0.39<br>(24) | 0.14   | 0.25  | 0.12<br>(24) | 0.21  | 0.04  |
| Colusa       | 5.92<br>(32)     | 0.19<br>(9)  | 0.27   | 0.07  | 0.34<br>(9)  | 0.13  | 0.24  |
| Contra Costa | 0.35<br>(35)     | 0.0<br>(5)   | 0.0    | 0.0   | 0.0<br>(5)   | 0.0   | 0.0   |
| Fresno       | 1.24<br>(35)     | 0.0<br>(13)  | 0.19   | 1.45  | 0.0<br>(13)  | -0.20 | -0.17 |
| Glenn        | 9.96<br>(34)     | 0.07<br>(10) | 0.25   | 0.13  | 0.24<br>(10) | 0.54  | -0.11 |
| Imperial     | 2.72<br>(35)     | 1.15<br>(30) | 0.38   | 0.0   | 0.17<br>(26) | -0.06 | 0.0   |
| Kern         | 2.80<br>(35)     | 0.94<br>(30) | 0.64   | 0.99  | 0.16<br>(29) | -0.17 | -0.05 |
| Kings        | 2.31<br>(27)     | 1.45<br>(9)  | 1.15   | 0.19  | 0.28<br>(6)  | 0.46  | -0.85 |
| Los Angeles  | 0.17<br>(25)     | 0.0<br>(3)   | 0.0    | 0.0   | 0.0<br>(3)   | 0.0   | 0.0   |
| Madera       | 0.45<br>(32)     | 0.83<br>(8)  | 0.87   | 0.0   | 0.0<br>(6)   | -0.08 | 0.0   |
| Marin-Sonoma | 0.41<br>(35)     | 0.0<br>(9)   | 0.0    | 0.0   | 0.0<br>(9)   | 0.0   | 0.0   |

Table IV-13. (cont'd)

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| County          | LTI          | WEEMIR       | TURMIR | HPMIR | WEE           | TUR   | HP    |
|-----------------|--------------|--------------|--------|-------|---------------|-------|-------|
| Merced          | 2.24<br>(35) | 0.0<br>(16)  | 0.23   | 0.23  | 0.0<br>(16)   | -0.02 | -0.02 |
| Orange          | 0.20<br>(28) | 0.0<br>(10)  | 1.69   | 0.0   | 0.0<br>(7)    | -0.37 | 0.0   |
| Placer          | 0.35<br>(7)  | 0.15<br>(11) | 0.52   | 0.34  | 0.0<br>(4)    | 0.0   | 0.75  |
| Riverside       | 1.51<br>(35) | 1.25<br>(33) | 0.34   | 0.13  | -0.08<br>(33) | 0.41* | 0.28  |
| Sacramento-Yolo | 2.72<br>(33) | 0.38<br>(20) | 0.31   | 0.22  | -0.28<br>(20) | -0.20 | -0.16 |
| San Bernardino  | 2.39<br>(15) | 1.13<br>(20) | 1.83   | 0.11  | -0.12<br>(9)  | 0.35  | 0.0   |
| San Diego       | 0.51<br>(21) | 0.0<br>(13)  | 0.78   | 3.11  | 0.0<br>(8)    | 0.07  | 0.73  |
| San Joaquin     | 1.19<br>(35) | 0.25<br>(10) | 0.16   | 0.36  | 0.25<br>(10)  | 0.08  | 0.07  |
| Santa Clara     | 1.14<br>(33) | 0.0<br>(4)   | 0.0    | 0.0   | 0.0<br>(4)    | 0.0   | 0.0   |
| Shasta          | 2.24<br>(35) | 0.59<br>(14) | 1.14   | 0.07  | 0.10<br>(14)  | 0.03  | -0.11 |

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Table IV-13. (cont'd)

| County      | LTI           | WEEMIR        | TURMIR | HPMIR | WEE            | TUR   | HP     |
|-------------|---------------|---------------|--------|-------|----------------|-------|--------|
| Solano      | 0.41<br>(35)  | 0.0<br>(7)    | 0.18   | 0.0   | 0.0<br>(7)     | -0.33 | 0.0    |
| Stanislaus  | 1.51<br>(35)  | 0.51<br>(16)  | 0.23   | 1.21  | -0.38<br>(16)  | 0.07  | 0.01   |
| Tehama      | 1.69<br>(31)  | 0.22<br>(12)  | 0.69   | 0.41  | 0.22<br>(12)   | 0.26  | -0.19  |
| Tulare      | 0.51<br>(35)  | 1.42<br>(23)  | 0.23   | 3.07  | 0.13<br>(23)   | 0.08  | 0.09   |
| Sutter-Yuba | 5.03<br>(33)  | 0.33<br>(22)  | 1.01   | 0.11  | 0.08<br>(21)   | 0.50* | 0.26   |
| State Wide  | 1.40<br>(958) | 0.59<br>(407) | 0.53   | 0.63  | -0.01<br>(350) | 0.01  | -0.11* |

\* Significant at the 0.05 level.  
[a] number of observations.



Table IV-14. Correlation (r) (Pearson product moment) of monthly western equine encephalomyelitis and Turlock virus minimum infection rates (MIR) in *Cx. tarsalis* with the percent of sentinel chickens (TURCHK and WEECHK) that seroconverted to Turlock and western equine encephalomyelitis viruses in California counties, 1978-1982.

| County          | Mean          |              |        |              |        | r             |       |
|-----------------|---------------|--------------|--------|--------------|--------|---------------|-------|
|                 | LTI           | TURMIR       | WEEMIR | TURCHK       | WEECHK | TUR           | WEE   |
| Butte           | 14.85<br>(35) | 0.14<br>(24) | 0.40   | 9.05<br>(25) | 3.28   | 0.23<br>(22)  | 0.58* |
| Colusa          | 5.92<br>(32)  | 0.26<br>(9)  | 0.18   | 6.36<br>(15) | 4.93   | -0.27<br>(7)  | 0.0   |
| Fresno          | 1.24<br>(35)  | 0.19<br>(13) | 0.0    | 4.87<br>(21) | 7.32   | 0.0<br>(9)    | 0.0   |
| Glenn           | 9.96<br>(34)  | 0.25<br>(10) | 0.07   | 6.09<br>(10) | 7.12   | 0.0<br>(0)    | 0.0   |
| Imperial        | 2.72<br>(35)  | 0.38<br>(30) | 1.15   | 3.18<br>(20) | 15.37  | -0.01<br>(15) | 0.30  |
| Kern            | 2.80<br>(35)  | 0.64<br>(30) | 0.94   | 9.50<br>(26) | 8.36   | -0.16<br>(26) | 0.68* |
| Kings           | 2.31<br>(27)  | 1.15<br>(9)  | 1.45   | 3.44<br>(5)  | 23.50  | 0.0<br>(1)    | 0.0   |
| Merced          | 2.24<br>(35)  | 0.23<br>(16) | 0.0    | 5.15<br>(16) | 0.0    | -0.29<br>(7)  | 0.0   |
| Placer          | 0.35<br>(7)   | 0.52<br>(11) | 0.15   | 6.01<br>(15) | 5.37   | 0.94*<br>(8)  | 0.23  |
| Riverside       | 1.51<br>(35)  | 0.34<br>(33) | 1.25   | 3.28<br>(20) | 1.31   | -0.02<br>(20) | 0.40  |
| Sacramento-Yolo | 2.72<br>(33)  | 0.31<br>(20) | 0.38   | 3.20<br>(10) | 0.0    | -0.22<br>(8)  | 0.0   |
| San Bernardino  | 2.39<br>(15)  | 1.83<br>(20) | 1.14   | 0.91<br>(10) | 0.45   | -0.23<br>(8)  | 0.0   |
| San Diego       | 0.51<br>(21)  | 0.78<br>(13) | 0.0    | 0.0<br>(9)   | 0.0    | 0.0<br>(5)    | 0.0   |

Table IV-14. (cont'd)

| County      | LTI           | TURMIR        | WEEMIR | TURCHK        | WEECHK | TUR            | WEE   |
|-------------|---------------|---------------|--------|---------------|--------|----------------|-------|
| San Joaquin | 1.19<br>(35)  | 0.16<br>(10)  | 0.25   | 11.55<br>(10) | 0.0    | -0.52<br>(3)   | 0.0   |
| Shasta      | 2.24<br>(35)  | 1.14<br>(14)  | 0.59   | 2.81<br>(21)  | 2.36   | 0.78*<br>(12)  | 0.52  |
| Tehama      | 1.69<br>(31)  | 0.69<br>(12)  | 0.22   | 15.92<br>(15) | 3.71   | 0.83*<br>(15)  | 0.0   |
| Tulare      | 0.51<br>(35)  | 0.23<br>(23)  | 1.42   | 1.18<br>(21)  | 1.57   | 0.40<br>(18)   | 0.48* |
| Sutter-Yuba | 5.03<br>(33)  | 1.01<br>(22)  | 0.33   | 8.84<br>(25)  | 3.17   | 0.12<br>(20)   | 0.71* |
| State Wide  | 1.40<br>(958) | 0.53<br>(407) | 0.59   | 5.98<br>(289) | 4.69   | 0.18*<br>(200) | 0.33* |

\* Significant at the 0.05 level.  
 [a] Number of observations.

Table IV-15. Multiple linear regression model for Hart Park virus in the Sacramento Valley (Glenn, Butte, Sutter-Yuba) and Kern County California. Dependent variable, Hart Park minimum infection rate; independent variables entered into the equation for selection of best 5 variable model were URBLTI, RURLTI, TMEAN, TO, TMAX, RAIN, TOTTO, TOTTMAX, TOTRAIN, HRSLITE, CHBEN, CHMMO. Computer program selected the 5 variables that explained the maximum amount of variation of the dependent variable.

| Variable         | B [a]   | S.E.<br>B | P<br>(B NE 0) | Model     |       |
|------------------|---------|-----------|---------------|-----------|-------|
|                  |         |           |               | R-squared | P [b] |
| -----            |         |           |               |           |       |
| Sac Valley, N=22 |         |           |               |           |       |
| URBLTI           | 2.2591  | 0.7568    | .0087         | 0.47      | .0523 |
| TOTRAIN          | 0.0020  | 0.0008    | .0175         |           |       |
| TOTTO            | 0.0026  | 0.0010    | .0220         |           |       |
| TOTTMAX          | -0.0123 | 0.0077    | .1288         |           |       |
| CHMMO            | 2.3788  | 0.7965    | .0087         |           |       |
| Kern, N=29       |         |           |               |           |       |
| URBLTI           | -3.8628 | 1.8721    | 0.0505        | 0.48      | .0072 |
| TMAX             | 0.0130  | 0.0093    | 0.1716        |           |       |
| TOTTO            | 0.0015  | 0.0007    | 0.0377        |           |       |
| TOTTMAX          | -0.0110 | 0.0058    | 0.0708        |           |       |
| HRSLITE          | 0.6107  | 0.4331    | 0.1719        |           |       |

[a] Partial regression coefficient.

[b] Probability that the variation explained by the 5 variable model was greater than expected by chance alone.

Table IV-16. Multiple linear regression model for Turlock virus in the Sacramento Valley (Glenn, Butte, Sutter-Yuba) and Kern County California. Dependent variable, Turlock minimum infection rate; independent variables entered into the equation for selection of best 5 variable model were URBLTI, RURLTI, TMEAN, TO, TMAX, RAIN, TOTTO, TOTTMAX, TOTRAIN, HRSLITE, CHBEN, CHMMO. Computer program selected the 5 variables that explained the maximum amount of variation of the dependent variable.

| Variable         | B [a]   | S.E.<br>B | P<br>(B NE O) | Model     |       |
|------------------|---------|-----------|---------------|-----------|-------|
|                  |         |           |               | R-squared | P [b] |
| -----            |         |           |               |           |       |
| Sac Valley, N=22 |         |           |               |           |       |
| RURLTI           | 0.4370  | 0.4702    | 0.3665        | 0.36      | .1713 |
| TMAX             | 0.0179  | 0.0113    | 0.1338        |           |       |
| TOTRAIN          | -0.0013 | 0.0100    | 0.2163        |           |       |
| TOTTMAX          | -0.0078 | 0.0085    | 0.3687        |           |       |
| HRSLITE          | -0.2991 | 0.3921    | 0.4567        |           |       |
| Kern, N=29       |         |           |               |           |       |
| RURLTI           | -1.5155 | 1.0516    | 0.1630        | 0.32      | .0981 |
| MEANTEMP         | 0.2921  | 0.1508    | 0.0651        |           |       |
| RAIN             | 0.0665  | 0.0335    | 0.0592        |           |       |
| TMAX             | -0.0148 | 0.0114    | 0.2056        |           |       |
| TOTRAIN          | -0.0019 | 0.0012    | 0.1476        |           |       |

[a] Partial regression coefficient.

[b] Probability that the variation explained by the 5 variable model was greater than expected by chance alone.

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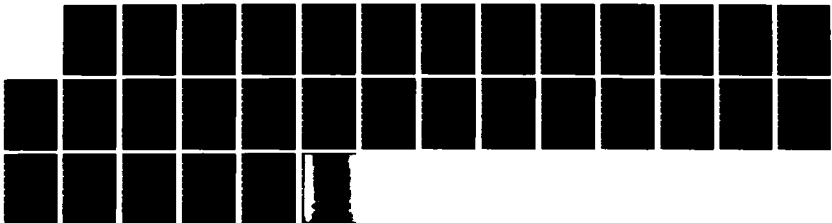
AN EPIDEMIOLOGICAL INVESTIGATION OF HART PARK AND  
TURLOCK VIRUSES IN CALIFORNIA(U) AIR FORCE INST OF TECH  
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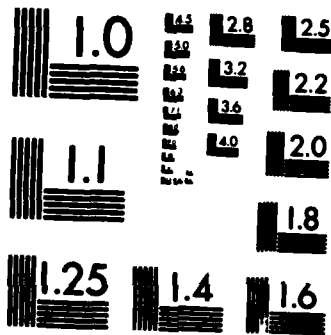
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Table IV-17. Multiple linear regression model for WEE virus in the Sacramento Valley (Glenn, Butte, Sutter-Yuba) and Kern County California. Dependent variable, western equine encephalomyelitis minimum infection rate; independent variables entered into the equation for selection of best 5 variable model were URBLTI, RURLTI, TMEAN, TO, TMAX, RAIN, TOTTO, TOTTMAX, TOTRAIN, HRSLITE, CHBEN, CHMMO. Computer program selected the 5 variables that explained the maximum amount of variation of the dependent variable.

| Variable         | B [a]   | S.E.<br>B | P<br>(B NE O) | Model     |       |
|------------------|---------|-----------|---------------|-----------|-------|
|                  |         |           |               | R-squared | P [b] |
| -----            |         |           |               |           |       |
| Sac Valley, N=22 |         |           |               |           |       |
| RURLTI           | -0.8445 | 0.5191    | 0.1233        | 0.30      | .2907 |
| URBLTI           | 2.6370  | 1.2423    | 0.0498        |           |       |
| RAIN             | 0.0143  | 0.0155    | 0.3700        |           |       |
| TOTTMAX          | 0.0063  | 0.0092    | 0.5003        |           |       |
| TOTTO            | -0.0006 | 0.0006    | 0.5003        |           |       |
| Kern, N=29       |         |           |               |           |       |
| RAIN             | 0.1138  | 0.0302    | 0.0010        | 0.74      | .0001 |
| TMAX             | -0.0121 | 0.0068    | 0.0923        |           |       |
| HRSLITE          | 1.6875  | 0.3963    | 0.0003        |           |       |
| CHMMO            | -7.4046 | 2.1364    | 0.0021        |           |       |
| CHBEN            | 5.7843  | 2.1814    | 0.0143        |           |       |

[a] Partial regression coefficient.

[b] Probability that the variation explained by the 5 variable model was greater than expected by chance alone.

Table IV-18. Comparison of isolations of Turlock and Hart Park viruses from male and female Cx. tarsalis in Kern County, 1983.

| Sex     |              | Location and number |               |              |              | Total  |
|---------|--------------|---------------------|---------------|--------------|--------------|--------|
|         |              | Breck-<br>enridge   | Kern<br>River | Poso<br>West | John<br>Dale |        |
| Males   | TUR isolates | 0                   | 0             | 0            | 0            | 0 [a]  |
|         | HP isolates  | 1 [b]               | 0             | 0            | 0            | 1 [c]  |
|         | No. tested   | 4,335               | 2,610         | 719          | 1,184        | 8,848  |
| Females | TUR isolates | 0                   | 9             | 4            | 12           | 25     |
|         | HP isolates  | 19                  | 37            | 12           | 44           | 112    |
|         | No. tested   | 6,787               | 14,188        | 5,430        | 11,964       | 38,369 |

[a] Fisher's exact test indicates that TUR isolation rates from males and females were different ( $p=0.00558$ ).

[b] Fisher's exact test indicates that HP isolation rates from males and females were different ( $p=0.00004$ ).

[c] Fisher's exact test indicates that HP isolation rates from males and females were different ( $p<<0.00001$ ) (uncorrected for multiple comparisons).



FIGURES

Figure II-1. Growth of Hart Park (AR70) virus in Cx. tarsalis incubated at 27 C following intrathoracic inoculation. Mean (log pfu/mosquito) titer determined from tests on 10 individual mosquitoes at each time post inoculation by assay on Vero cells. Plotted as mean  $\pm$  1 standard deviation.

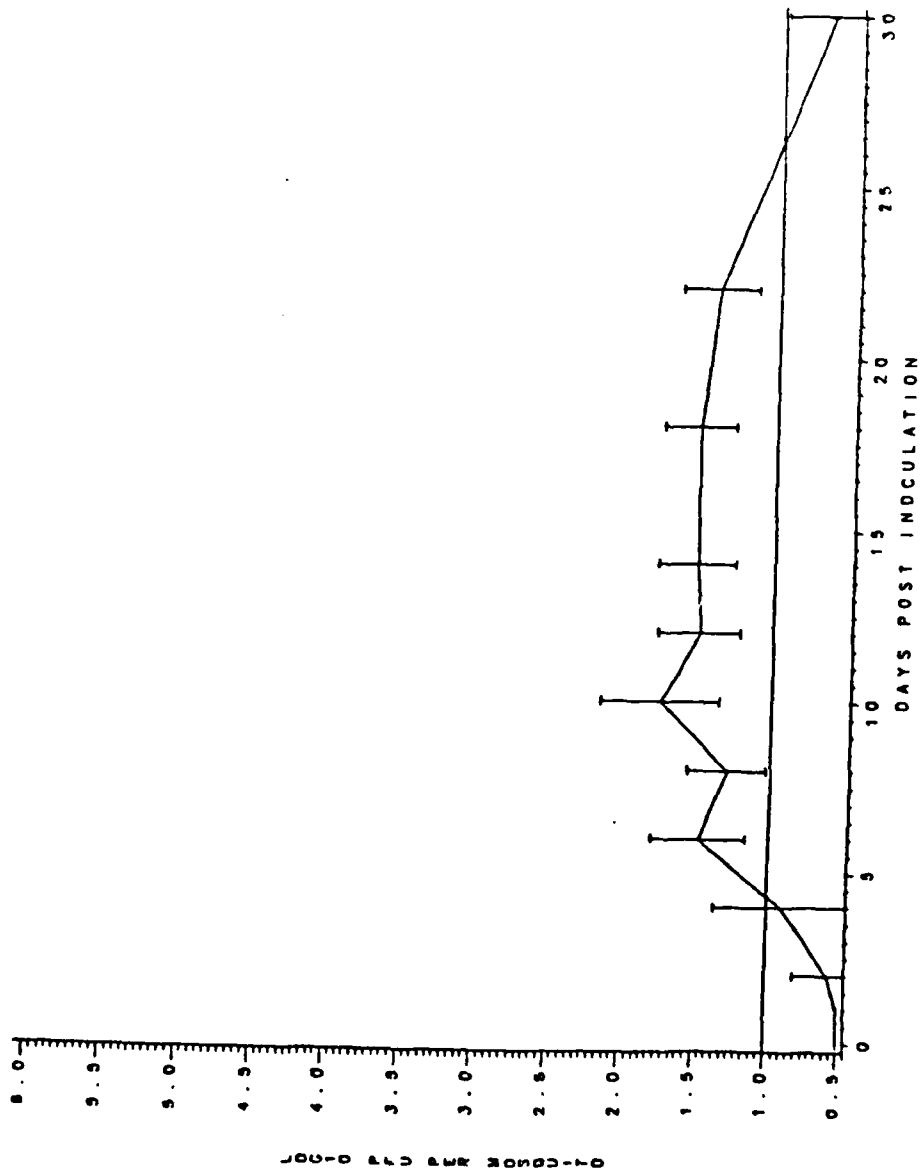


Figure II-2. Growth of Hart Park (BFN5662) virus in Cx. tarsalis incubated at 27 C following intrathoracic inoculation. Mean titer (log pfu/mosquito) determined from tests on 10 individual mosquitoes at each time post inoculation by assay on Vero cells. Plotted as mean  $\pm$  1 standard deviation.

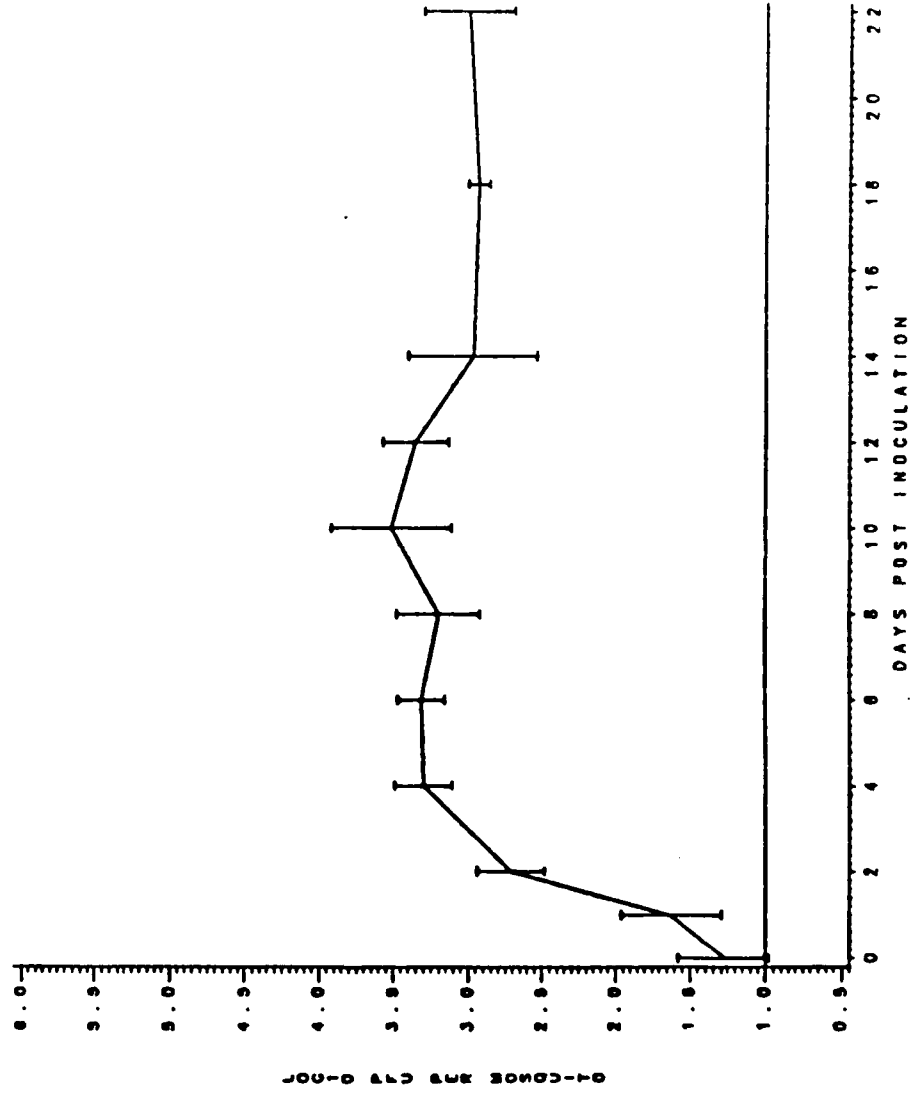


Figure II-3. Growth of Hart Park (BFNS662) virus in Cx. tarsalis after intrathoracic inoculation and incubation at 18 C. Mean (log pfu/mosquito) titer determined from tests on 5 individual <sup>10</sup> mosquitoes at each time post inoculation by assay on Vero cells. Plotted as mean  $\pm$  1 standard deviation.

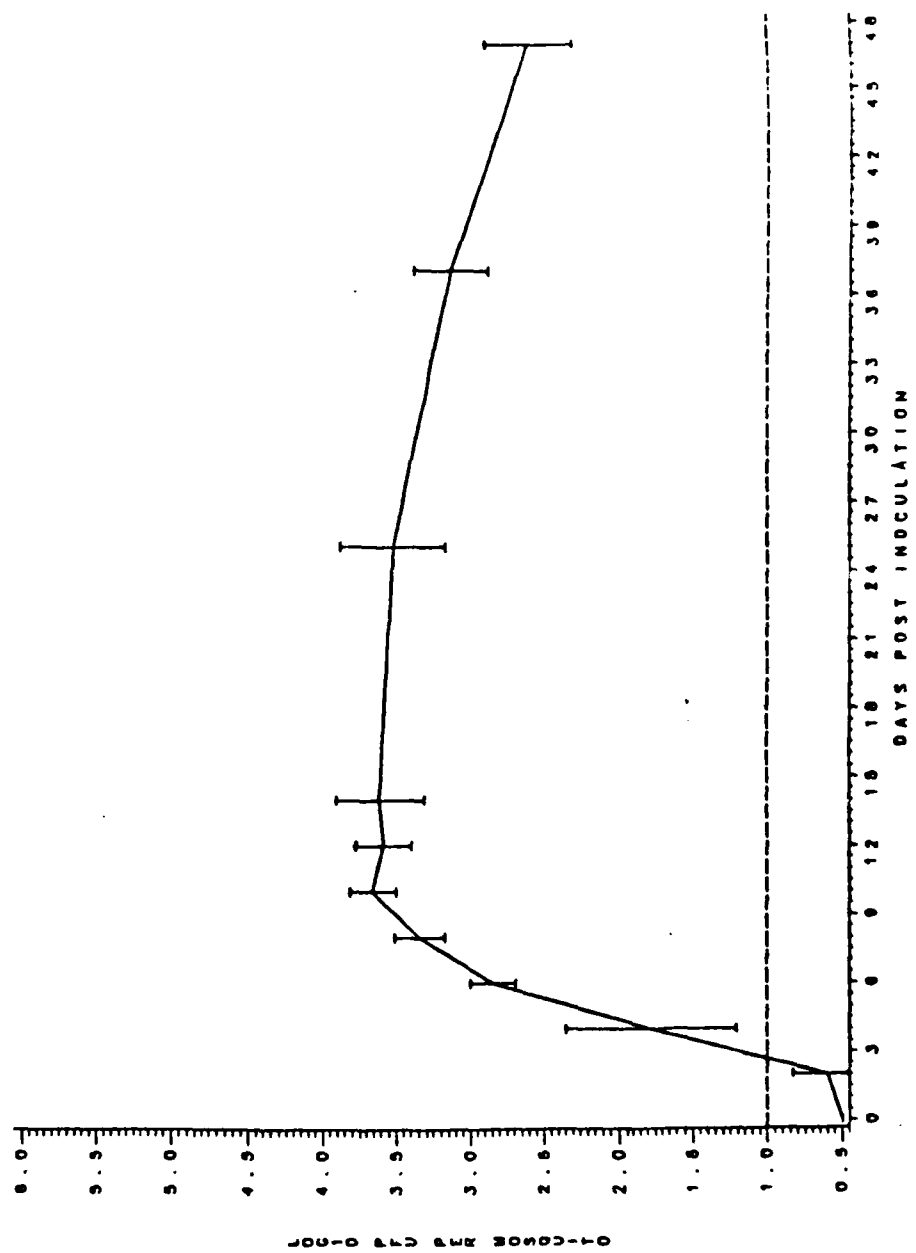


Figure II-4. Growth of Turlock (FMS4783) virus in Cx. tarsalis after intrathoracic inoculation and incubation at 18 C. Mean (log pfu/mosquito) titer determined from tests on 5 individual mosquitoes at each time post inoculation by assay on DECC. Plotted as mean  $\pm$  1 standard deviation.



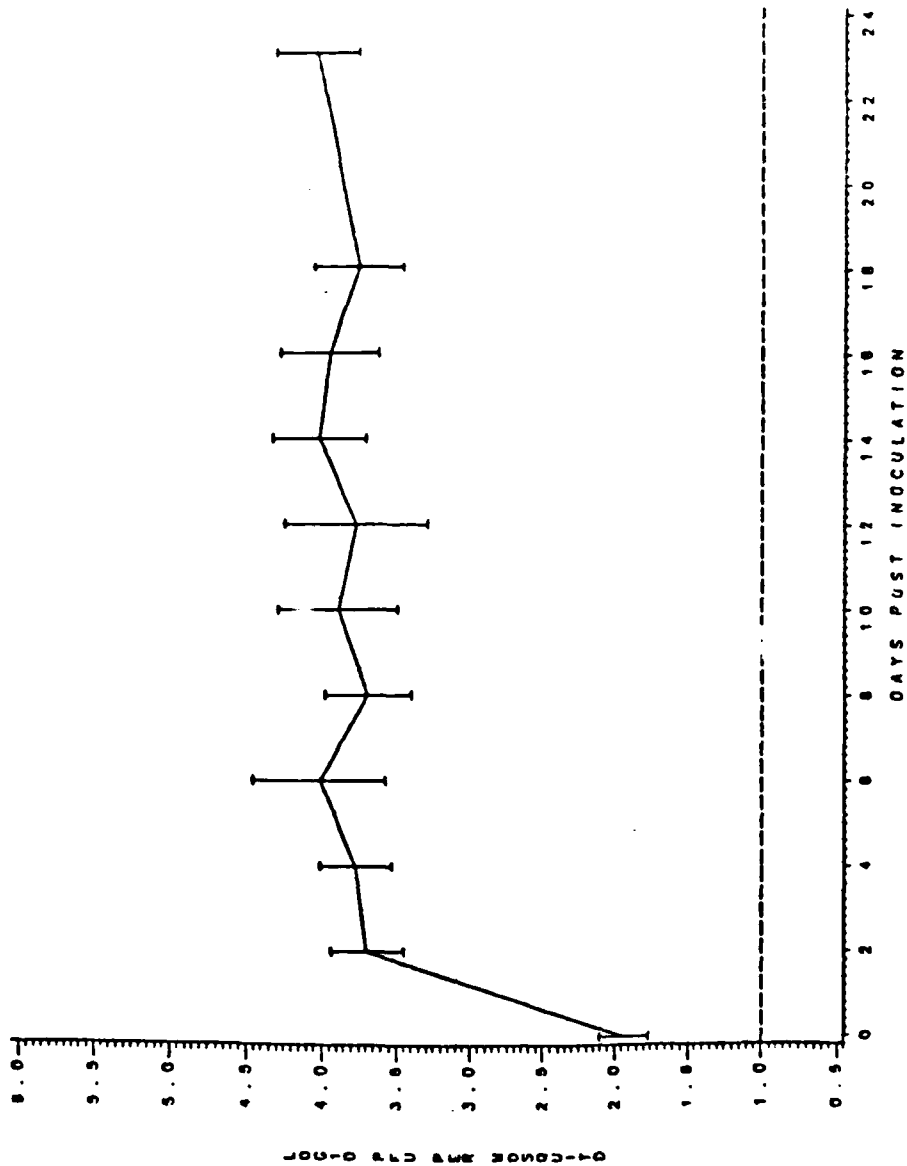


Figure II-5. Growth of Hart Park (BFN5662) virus in duck embryonic cells at 37 C. Mean ( $\log_{10}$  pfu/0.1ml) titer determined on 3 individual tubes of cells at each of the indicated days post inoculation. Plotted as mean  $\pm$  1 standard deviation.

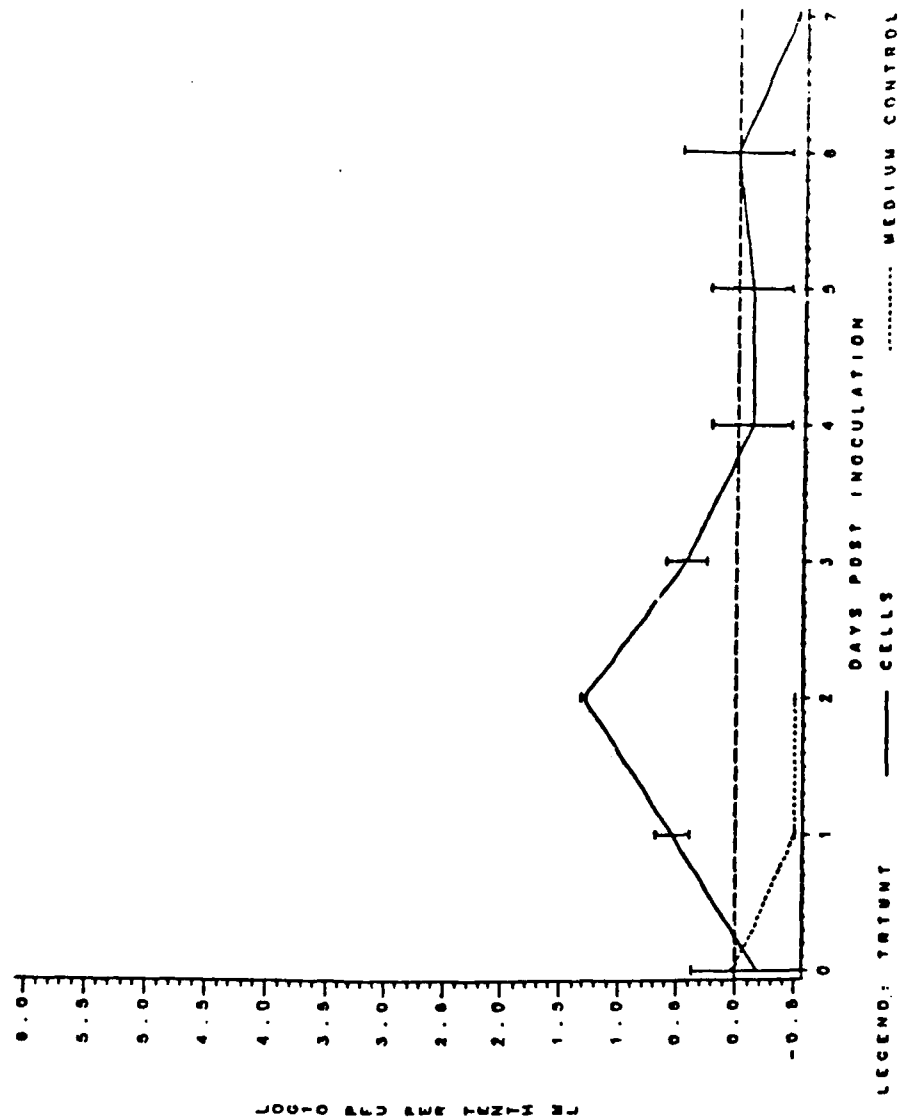


Figure II-6. Growth of Hart Park (BFNS662) virus in Vero cells at 37 C. Mean ( $\log_{10}$  pfu/0.1ml) titer determined on 3 individual tubes of cells at each of the indicated days post inoculation. Plotted as mean  $\pm$  1 standard deviation.

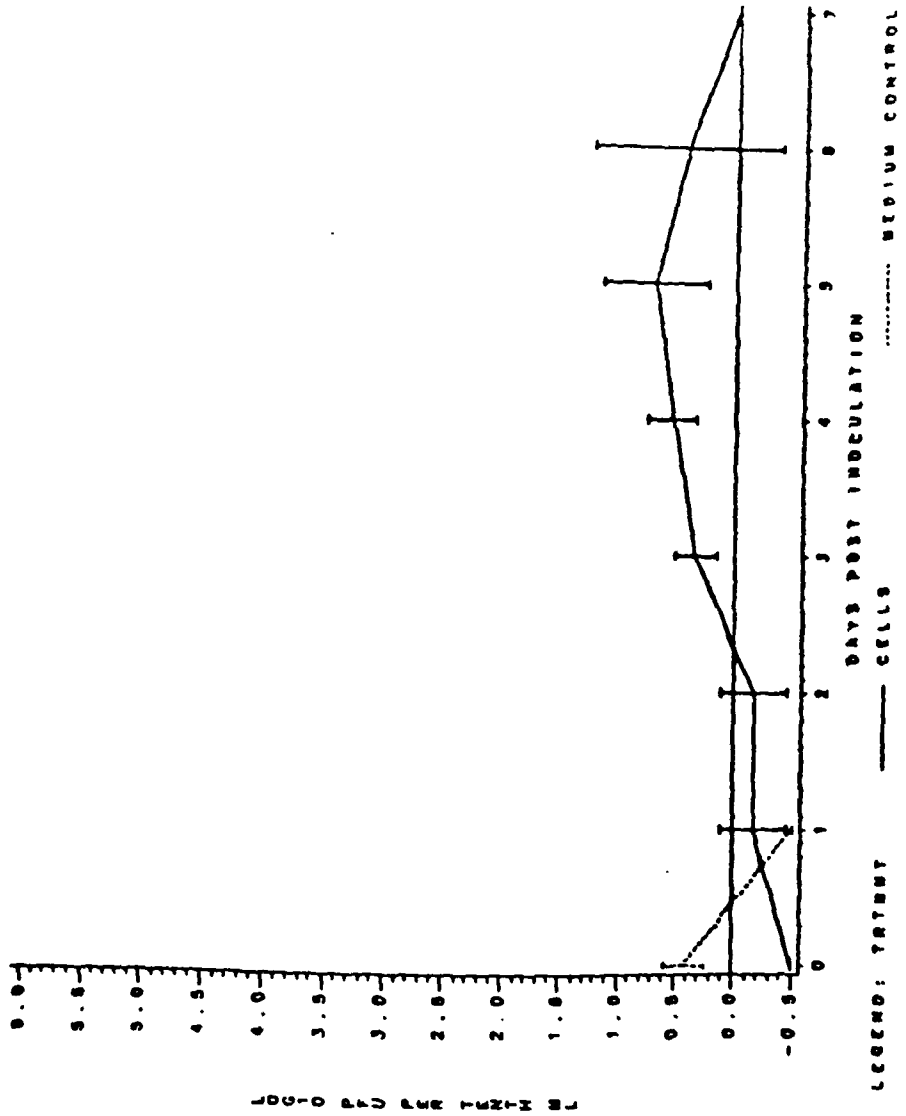


Figure II-7. Growth of Hart Park (BFN5662) virus in BHK (0853) cells at 37 C. Mean ( $\log_{10}$  pfu/0.1ml) titer determined on 3 individual tubes of cells at each of the indicated days post inoculation. Plotted as mean  $\pm$  1 standard deviation.

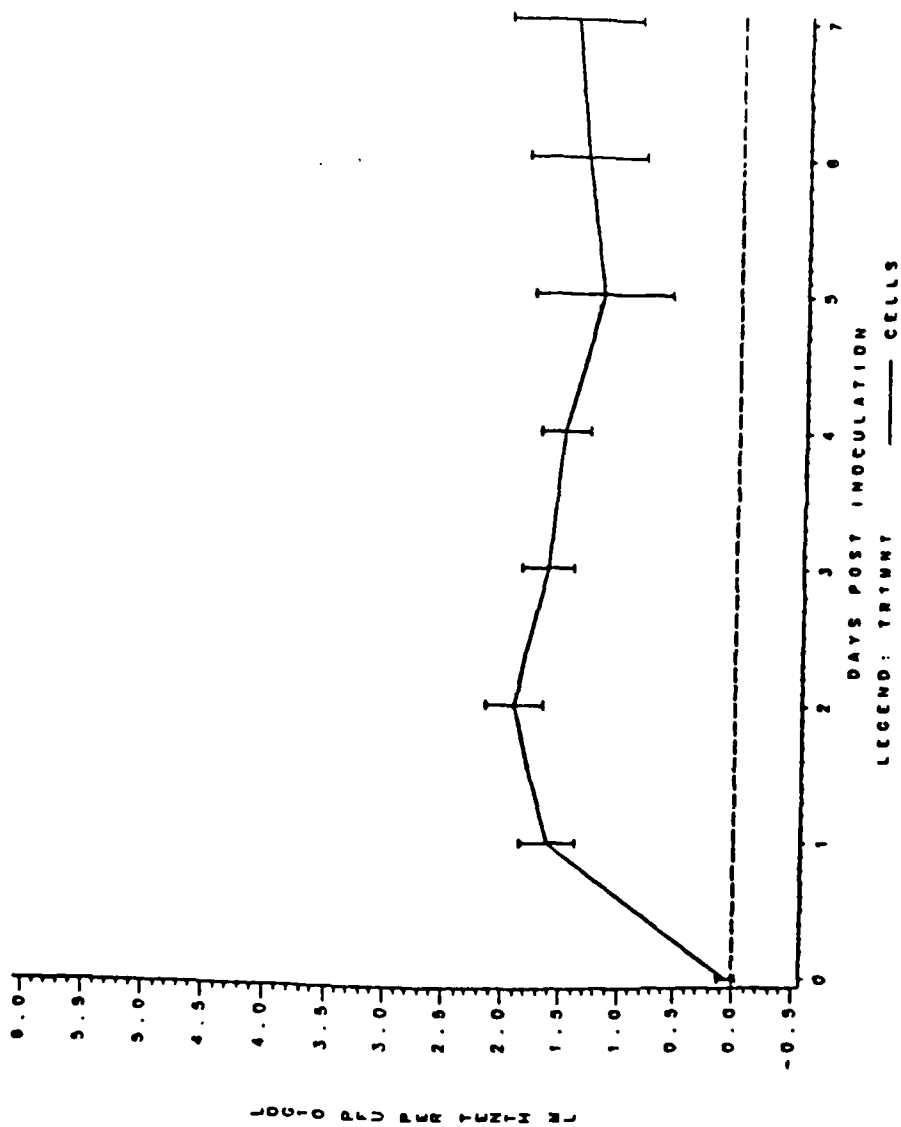


Figure II-8. Growth of Hart Park (BFN5662) virus in Aedes albopictus (C6/36) cells at 28 C. Mean ( $\log_{10}$  pfu/0.1ml) titer determined on 3 individual tubes of cells at each of the indicated days post inoculation. Plotted as mean  $\pm$  1 standard deviation.



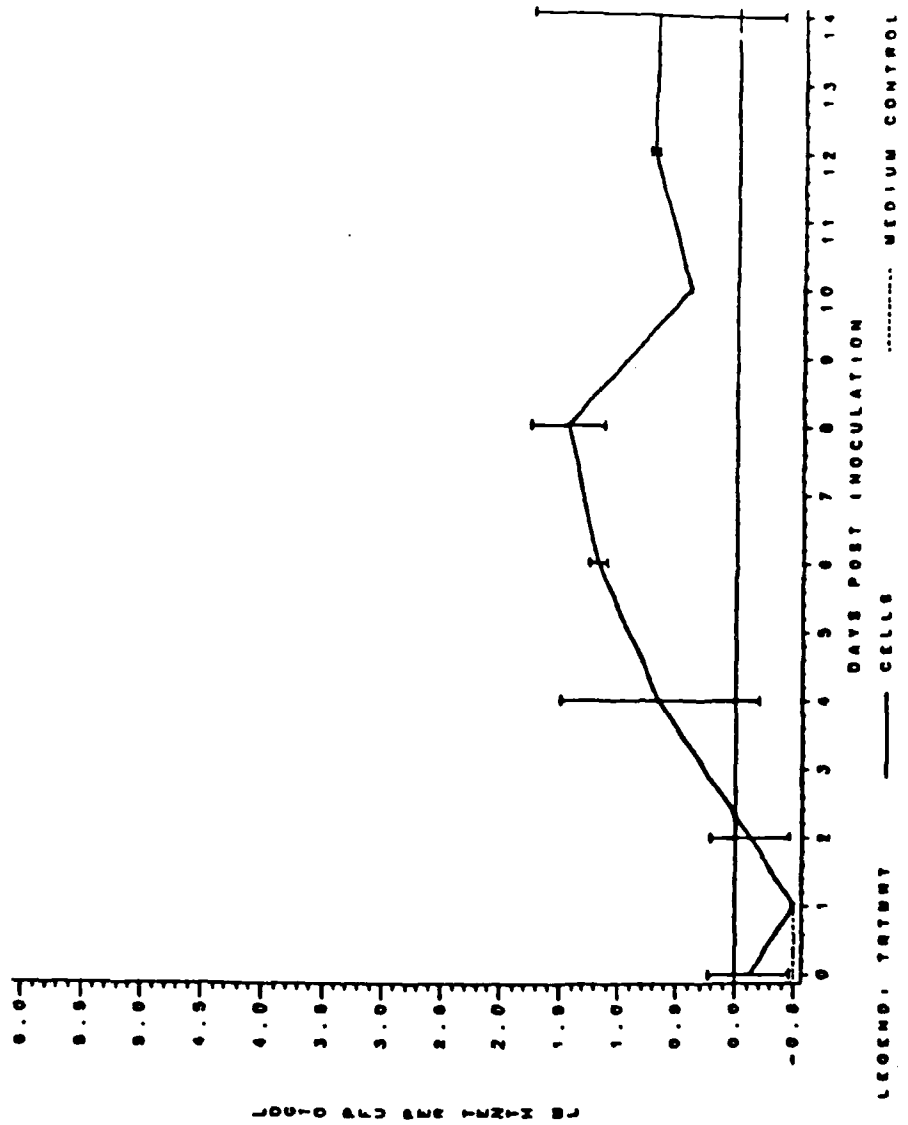


Figure II-9. Growth of Hart Park (BFN5662) virus in Cx. tarsalis cells at 28 C. Mean (log<sub>10</sub> pfu/0.1ml) titer determined on 3 individual tubes of cells at each of the indicated days post inoculation. Plotted as mean  $\pm$  1 standard deviation.

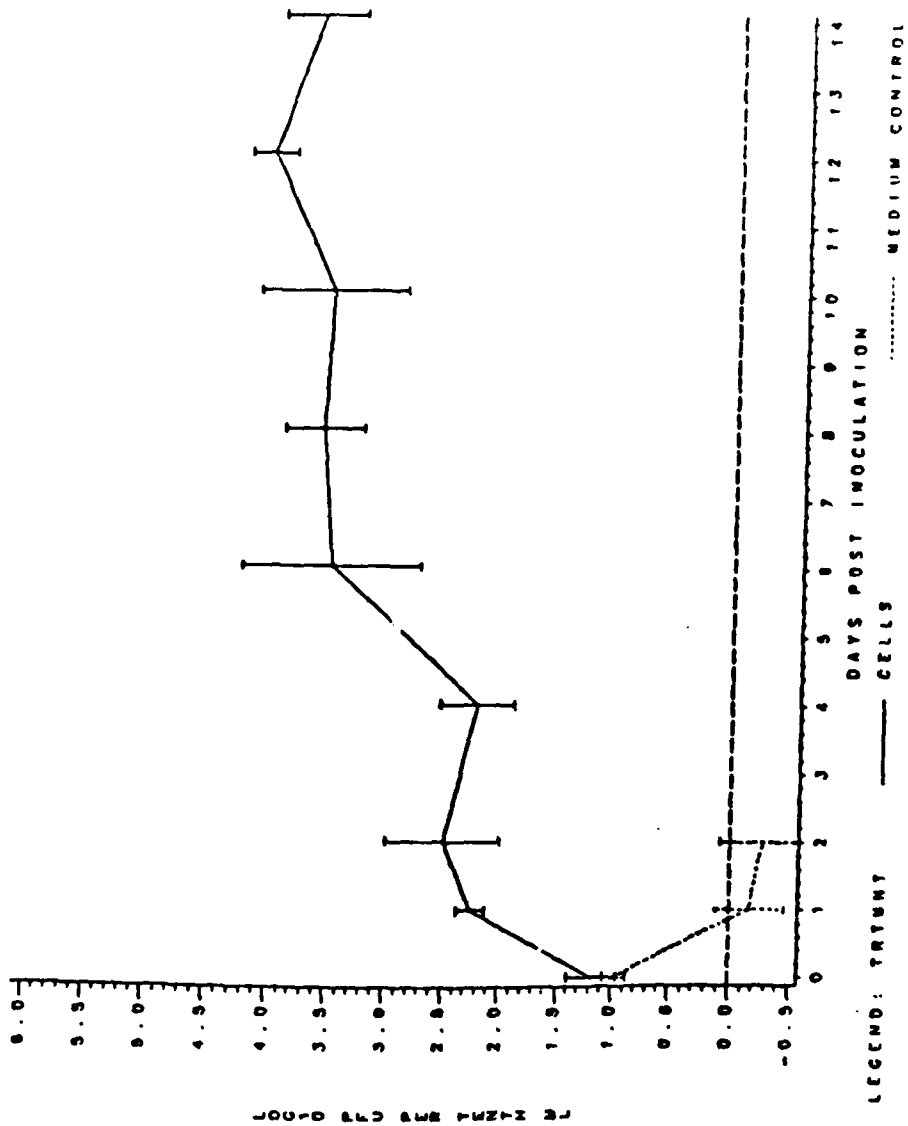


Figure II-10. Growth of Turlock (847-32) virus in duck embryonic cells at 37 C. Mean ( $\log_{10}$  pfu/0.1ml) titer determined on 3 individual tubes of cells at each of the indicated days post inoculation. Plotted as mean  $\pm$  1 standard deviation.

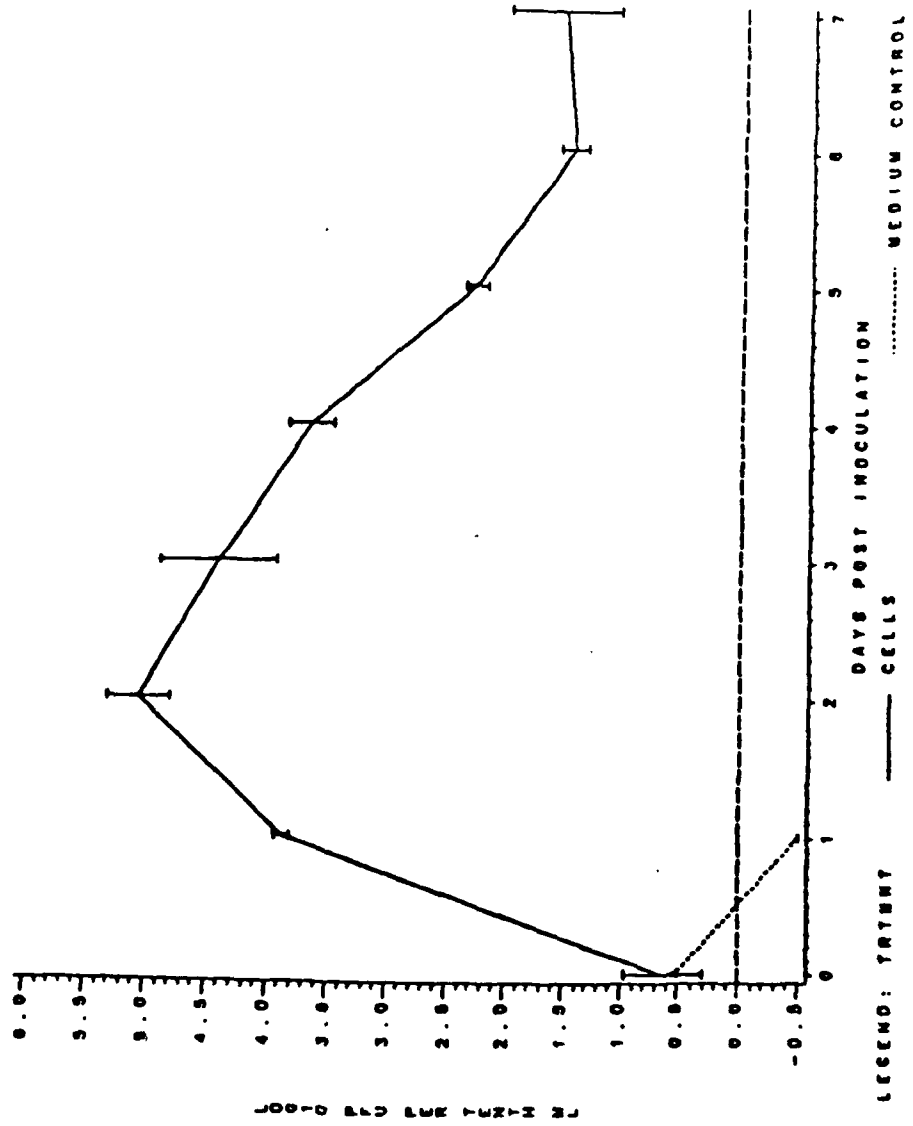


Figure II-11. Growth of Turlock (847-32) virus in Aedes albopictus (C6/36) cells at 28 C. Mean ( $\log_{10}$  pfu/0.1ml) titer determined on 3 individual tubes of cells at each of the indicated days post inoculation. Plotted as mean  $\pm$  1 standard deviation.

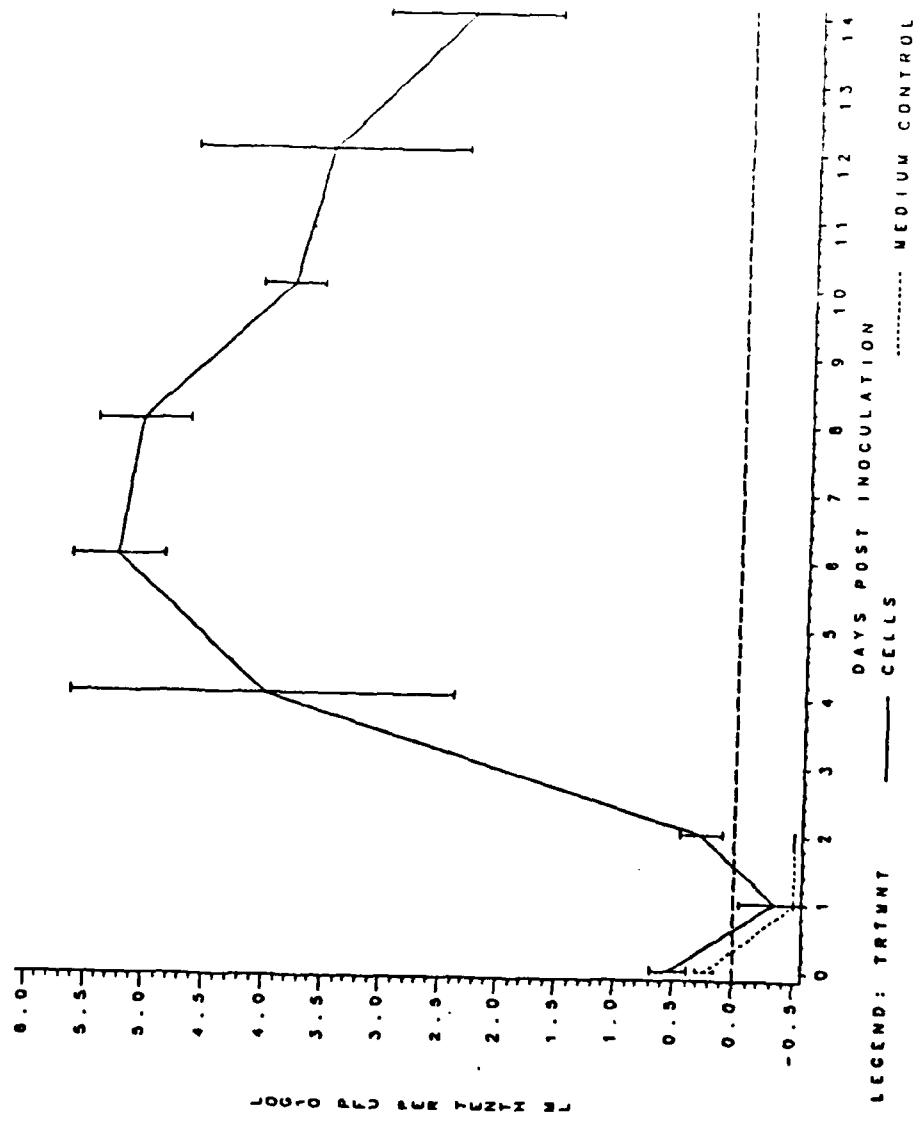


Figure II-12. Growth of Turlock (847-32) virus in Vero cells at 37  
C. Mean ( $\log_{10}$  pfu/0.1ml) titer determined on 3 individual tubes  
of cells at each of the indicated days post inoculation. Plotted  
as mean  $\pm$  1 standard deviation.



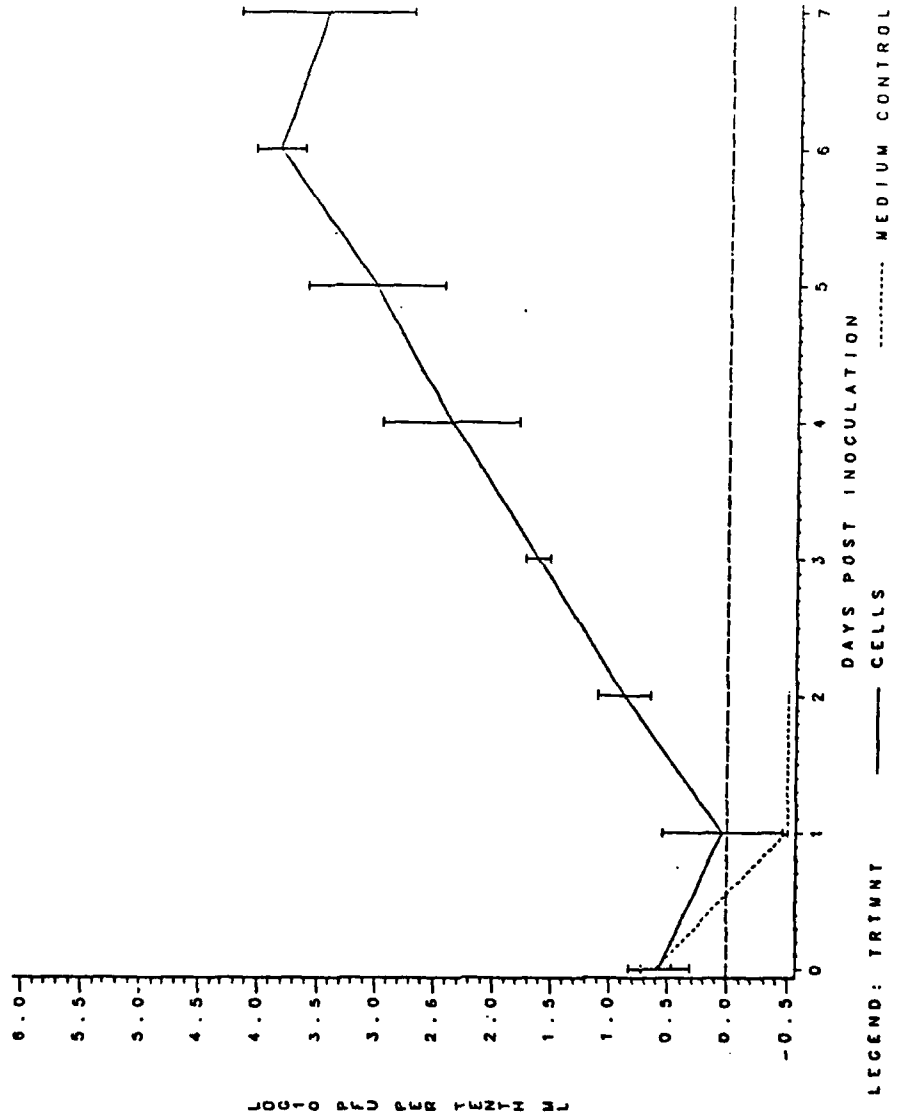


Figure V-1. Illustration of possible pathways of Hart Park viral transmission and persistence.

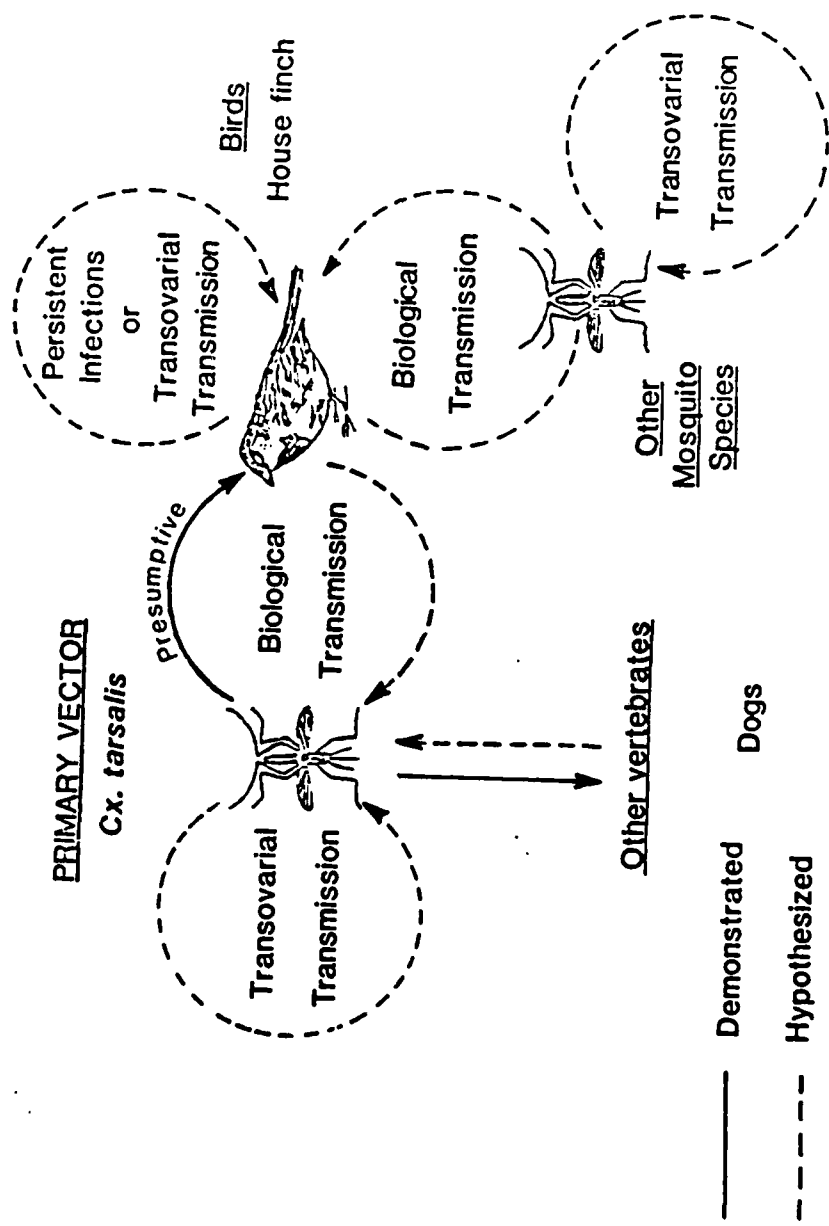
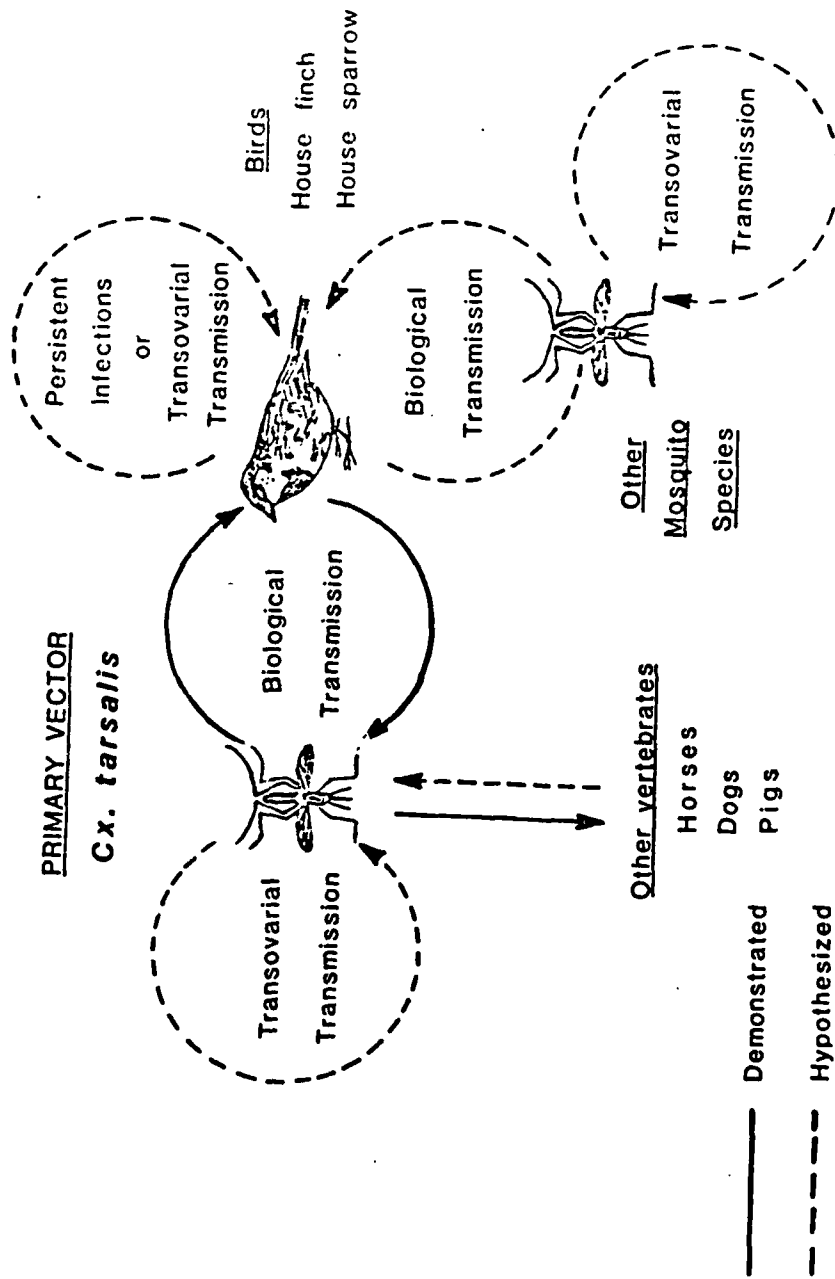


Figure V-2. Illustration of possible pathways of Turlock viral transmission and persistence.



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