BIOLOGICAL TRANSMISSION OF WESTERN ENCEPHALOMYELITIS VIRUS BY *CULEX TARSALIS COQUILLETT*

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ABSTRACT. A Manitoba strain of *Culex tarsalis* and a Manitoba isolate of western encephalomyelitis virus were used to determine vector ability under laboratory conditions. The mosquitoes were infected by feeding upon viremic day-old chicks. Secondary hosts were chicks and mice, and they were easily infected by the bite of a single infected mosquito. Transmission rates generally increased over 31 days to 100% by the end of the experiment. Infection rates remained at or near that level throughout the experiment. The infection threshold level was low, log 2.3 $TCID_{50}$. The combination of low infection threshold and high infection and transmission rates, indicates that *Cx. tarsalis* is highly susceptible to WE infection and can serve as an effective vector of WE in Manitoba.

INTRODUCTION

In Manitoba, western encephalomyelitis (WE) virus has been isolated from *Culex restuans* Theobald (Norris 1946) and *Cx. tarsalis* (McLintock 1947). Subsequent isolations of WE virus from mosquitoes have been reported (MacKay et al. 1968, Sekla and Stackiw 1976) but the infected species were not known. During a 1977 WE epidemic in Manitoba, WE virus was isolated from *Cx. tarsalis* collected from several locations in the province. WE virus was also isolated from *Aedes, Anopheles* and *Mansonia* mosquitoes (Sekla and Stackiw, pers. comm).

Cx. tarsalis is considered to be the main vector of WE in the western United States (Chamberlain et al. 1954a, Chamberlain and Sudia 1957, Hess and Holden 1958), and in Saskatchewan in Canada (McLintock et al. 1970). On purely ecological grounds, there is no reason to suspect that the situation is very different in Manitoba. In a 1975 epidemic, the peak of *Cx. tarsalis* populations in Winnipeg coincided with WE cases in horses and humans (Brust and Ellis 1976).

However, to incriminate a vector in the transmission of a disease organism, the vector must not only be present at the proper time and place and to be infected with the organism, but it must also be shown to be capable of transmitting the disease organism from one susceptible host to another susceptible host in the laboratory. Thus far, *Cx. tarsalis* in Manitoba has been incriminated on the basis of frequency of isolations and its presence in peak numbers during one epidemic. The present laboratory study was conducted to determine the infection threshold, the infection rate and the transmission rate of a Manitoba strain of *Cx. tarsalis* and a Manitoba isolate of WE. A vector potential gradient, based on the 3 criteria above, would indicate relative mosquito vector efficiency (Chamberlain et al. 1954b).

Infection threshold is considered to be the lowest concentration of virus capable of causing an infection in 50% of the mosquitoes ingesting it. The infection rate is the percentage of mosquitoes in a feeding series found to contain virus regardless of their ability to transmit it. The transmission rate is the percentage of mosquitoes transmitting the virus to susceptible animals. Biological transmission occurs after the virus has been incubated for several days within the mosquito, following an infected meal (Barnett 1956, Hayles et al. 1972, Thomas 1963).

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MATERIALS AND METHODS

VIRUS. The WE strain used in the experiments was originally isolated from a horse brain during the Manitoba epidemic of 1975. It had undergone 3 successive passages in Vero cell tissue cultures. Identity of the virus was confirmed by the National Arbovirus Reference Centre, Toronto, Canada. The titre of the stock virus was $\log_{10}$ 5.3 $TCID_{50}$ per 0.2 ml.

CELL CULTURE. Virus detection in mosquitoes, titrations of donor chick blood samples, and detection of virus in secondary hosts were done on Vero cells (green monkey kidney) which were originally obtained from the American Type Culture Collection. The growth medium was #1969 containing 10% bovine serum, 1% L-glutamine and antibiotics: 20,000 μg of penicillin, 10,000 μg of streptomycin, 0.05 μg of neomycin and 5,000 iu of mycostatin per 100 ml. It was buffered with 8% sodium bicarbonate and 10% HEPES solution.

The cells were routinely transferred by trypsinization with a 0.25% tryspin solution. Each plastic flask yielded a maximum of 50 tubes of Vero cells, each with 2 ml. of media cell suspension, for virus assay the following day. Infected cells were maintained in a medium equivalent to the growth medium, but with 1% bovine serum and no L-glutamine.

CHICKS. Day-old chicks were obtained from a local hatchery. Those used as donor chicks in the experiments on infection and transmission rates were inoculated intramuscularly (i.m.) with 0.03 ml. tissue culture fluid containing 1000 $TCID_{50}$ of virus, 15–18 hr before exposure to mosquitoes. Viremias were determined by taking blood samples from the chicks prior to and at the end of mosquito feedings. The length of the feeding period ranged between 15 and 27 hr after i.m. innoculation of the chicks.

The chicks used as secondary hosts were strapped to 2.5 × 15 mm cages, each containing 1 mosquito. These chicks were left exposed to the mosquitoes for 9 hr. All chicks that had been fed upon by mosquitoes were observed for 5 days. If death or CNS symptoms occurred within this 5-day period, the brains were removed for virus assay. Chicks showing no signs of infection within 5 days were considered to be negative.

The chicks used in the experiment on infection threshold were also inoculated with 0.03 ml. of tissue culture fluid containing 1000 $TCID_{50}$ of virus. Mosquito feeding commenced 3½ hr after chick infection and continued for 5 hr. At 1 hr intervals, the viremic chicks were strapped to cages containing mosquitoes and left there for 30 min. Immediately after each 30 min feeding, the chick was killed and exsanguinated, yielding a volume of blood no greater than 1 ml. The blood samples were used to determine the level of virus available to the mosquitoes.

To prevent coagulation, all chick blood samples were added to 2.5 ml of Alsevers solution containing twice the strength of antibiotics used in the cell culture growth medium. The blood samples were frozen at −70°C to preserve the virus and detach it from the red blood cells. After thawing, the blood samples were centrifuged at 4000 rpm for 20 min. The supernatant was used for virus titration.

MICE. The mice, used as secondary hosts in the transmission experiments, were obtained from disease-free colonies. Those mice that were fed upon by mosquitoes were kept for 10 days in isolation. If death occurred within this 10-day period, virus re-isolation from the brain was attempted to confirm infection. Otherwise, serology using the haemagglutination inhibition (HAI) test (Sekla and Stackiw 1976) was performed on the sera of surviving mice to detect antibody response from infection. Blood samples were centrifuged at 2000 rpm for 10 minutes and the serum removed and frozen for HAI testing.

MOSQUITOES. The mosquitoes used in these experiments were from the Manitoba colony of *Cx. tarsalis* kept at the Department of Entomology, University of Manitoba. All larvae were reared under a

photoperiod of L:D 16:8. The adults were maintained at the same photoperiod and at 75% relative humidity.

All experiments were conducted at 24°C. The mosquitoes were deprived of a sugar source at least 48 hours before they were allowed to feed upon infected chicks. Feeding was determined visually and all engorged females from each infection attempt were placed in separate cages, forming experimental groups. Oviposition dishes were supplied to these mosquitoes 4 and 5 days after the infective blood-meal. Mosquitoes were periodically removed from these groups for infectivity tests and/or transmission attempts to chicks or mice. An interval of at least 4 days elapsed before mosquitoes were allowed a second blood-meal.

In the experiment on transmission rate, mosquitoes were separately placed in 2.5 x 15 mm cages for individual feeding on secondary hosts. Mosquitoes that fed for the second time were harvested and stored for virus assay.

In the experiment on infection threshold, 5 groups of 30 mosquitoes were aspirated into 2.5 x 15 mm cages. All mosquitoes that fed during the 30 minutes that the chicks were strapped to the cages were kept for 12 days before preparation for virus assay.

RESULTS AND DISCUSSION

MOSQUITO INFECTION RATES. The WE infection rates in *Cx. tarsalis* are presented in Figure 1. The rates were high, remaining at or close to 100% throughout the incubation period of 31 days. The Manitoba strain of *Cx. tarsalis* is readily infected with WE virus. This corresponds closely to the results obtained by Chamberlain and Sudia (1957), and Hayles et al. (1972).

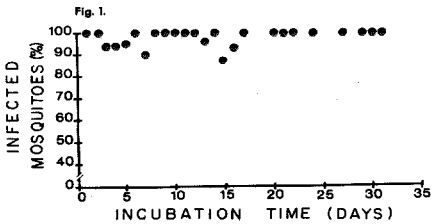

Figure 1. WE infection rates in *Culex tarsalis* at 24°C. Sample size 3–40/day.

Declines in the infection rates occurred on days 4–8 and 14–17. The small sampling size could account for these variations. However, declines in the rates within the 1st week may be due to virus inactivation before it had infected the mosquito and begun to multiply (Chamberlain et al. 1954a, Chamberlain and Sudia 1961).

Chamberlain and Sudia (1961) reported that mosquito infection rates of 100% are common in the laboratory. Such rates can be expected in almost any susceptible vector species provided the virus titre in the blood-meal is well above the threshold level, and incubation is sufficient for virus growth. A high infection rate alone is not proof of vector efficiency because the presence of virus in the mosquito does not guarantee that sufficient quantities to cause disease will be inoculated during feeding (McLintock et al. 1966). Infection rates are generally higher than transmission rates (Reeves et al. 1961).

Mosquitoes remained infected with WE throughout the length of the study, which lasted 31 days. This agrees with data obtained by other researchers who concluded that *Cx. tarsalis*, once infected, remains so for life (Barnett 1956, Chamberlain and Sudia 1961, Reeves and Hammon 1962). Such long-lived WE infections can be explained by continued progressive virus multiplication in various organs together with a slow virus mortality (Chamberlain and Sudia 1961).

MOSQUITO TRANSMISSION RATES. Transmission rates were high, indicating high infectivity of the mosquito's bite. *Cx. tarsalis* transmitted WE virus 126 times out of a possible 160 (79%) attempts to mice and chicks. There were 53 out of 70 (76%) successful transmissions to mice and 73 out of 90 (81%) to chicks (Figure

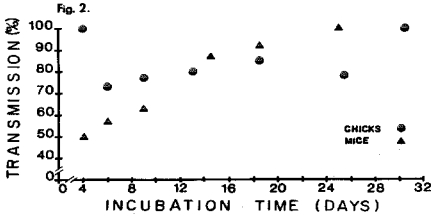

Figure 2. WE transmission rates by *Culex tarsalis* at 24°C. Sample size chicks 2–15, mice 1–14/day.

2). These transmissions occurred between days 4–31 post viral infection of the mosquitoes. Hayles et al. (1972) obtained 86% transmission rate to chicks at 24°C; very close to that found in this study. Other studies also indicate that *Cx. tarsalis* has a high efficiency of virus transmission (Chamberlain and Sudia 1957, Reeves and Hammon 1962).

Generally, the transmission rates to both hosts gradually increased to 100% as the incubation period lengthened. This gradual increase in transmission rates agrees with previous studies (Barnett 1956, Chamberlain and Sudia 1957, Hayles et al. 1972, Thomas 1963). Although it takes only a few days after virus ingestion for virus concentrations in mosquitoes to become high, it takes 2 to 3 times that incubation period for transmissions to become efficient. This is due to relocalization of the virus during incubation as well as an increase in the number of mosquitoes serving as vectors. The concentration in the salivary glands increases with a decrease elsewhere in the body of the mosquito (Chamberlain and Sudia 1961) and the number of mosquitoes with infected salivary glands increases with time following infection (Thomas 1963).

An initial high transmission rate, such as that which occurred on day 4 in the chick experiments may be due to mechanical transmission. Mechanical transmission causes a high transmission rate initially, then a gradual decline occurs as the virus dies off, until biological transmission takes over (Chamberlain and Sudia 1961). (However, Hayles et al. (1972) state that biological transmissions commence after incubation for 3 days.) Since transmissions to mice are lower, it is most likely that the high transmission rate (100%) to chicks on day 4 is a chance occurrence resulting from the small sample size (4).

The transmission experiments did not commence until the 4th day of incubation because this time period had been reported as the minimum extrinsic incubation period for WE virus in *Cx. tarsalis* (Barnett 1956, Hayles et al. 1972). Thomas (1963) showed that virus was not detected in the salivary glands until the fourth day. Also more mosquitoes were likely to feed at this time than before, and the chance of confusing mechanical transmission with biological transmission was reduced.

Since the extrinsic incubation period is short (4 days), many transmissions are possible. This is especially true since the virus is maintained in the mosquito's body for life. It is important that the length of the extrinsic incubation period coincides with the interval between blood-meals. This obviously indicates optimum adaptation by the organism to its vector.

MOSQUITO INFECTION THRESHOLD. The WE infection threshold in *Cx. tarsalis* is presented in Figure 3. Initially, there was

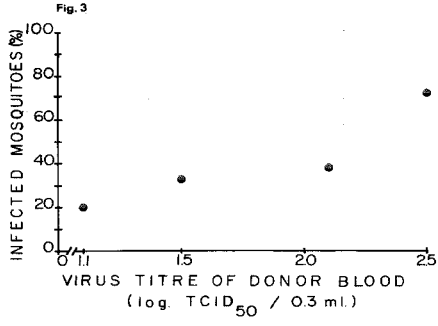

Figure 3. WE infection threshold in *Culex tarsalis* at 24°C. Sample size 5–18/test.

a slow increase in the number of mosquitoes infected with the increase in donor blood titre. After $\log_{10}2.1$ was reached a sharp rise occurred in the percentage of infected mosquitoes. By interpolating from the graph, a 50% infection level corresponds to a value slightly less than log 2.3 $TCID_{50}$/0.3 ml. of donor blood. This value corresponds closely to that found by Hayles et al. (1972) and Thomas (1963), who obtained threshold values of log 2.5 LD_{50}. Barnett (1956) and Hardy (1966) attained infective threshold values of log 3.0 LD_{50} and 3.2 LD_{50} respectively. The infective threshold values were obtained by measuring the viremia level at which mosquitoes would transmit the virus. The infection threshold merely measures the percentage of mosquitoes infected with virus, rather than their ability to transmit it. Higher values for the infective thresholds than for the infection thresholds would be expected if a critical level of virus in the mosquito is needed to infect the salivary glands. Thomas (1963) has shown that mosquitoes feeding upon donor chicks with low viremias may not have virus in the salivary glands, although the remainder of the body may be infected with virus.

The infection threshold found in this study is low when compared with other species infected with the same virus (Chamberlain et al. 1954b). Theoretically, mosquitoes with a lower infection threshold would be more effective vectors since lower levels of viremia in their hosts could result in infection of the mosquito and subsequent transmission. In addition to differences in infection threshold in different mosquito species, there may be differences in susceptibility to virus infection between field and laboratory populations of the same species (Hardy et al. 1976). Differences were also noted between individuals within the same population.

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