Effect of Environmental Temperature on the Ability of *Culex pipiens* (Diptera: Culicidae) to Transmit West Nile Virus

DAVID J. DOHM,¹ MONICA L. O'GUINN, AND MICHAEL J. TURELL

Department of Vector Assessment, Virology Division, United States Army Medical Research Institute of Infectious Diseases, 1425 Porter Street, Fort Detrick, MD 21702-5011


ABSTRACT Environmental temperature can affect the ability of mosquitoes to transmit an arbovirus. However, results of various studies indicate that these effects are not consistent among viruses or mosquito species, and there is no information available on the effect of environmental temperature on the ability of North American mosquito species to transmit West Nile (WN) virus. We evaluated the effect of incubation temperature (18, 20, 26, or 30°C) on the ability of *Culex pipiens* L. derived from specimens collected during the outbreak in New York in 1999 to transmit a strain of WN virus obtained from a crow that died during this outbreak. Although mosquitoes fed on the same viremic chickens, infection rates were directly related to subsequent incubation temperatures. In mosquitoes held at 30°C, virus was recovered from nearly all mosquitoes tested, disseminated infections were detected as early as 4 d after the infectious blood meal, and >90% of all mosquitoes had a disseminated infection 12 or more days after the infectious blood meal. In contrast, for mosquitoes held at 18°C, disseminated infections were not detected until 25 d after the infectious blood meal, and even after 28 d, <30% contained a disseminated infection. Results for mosquitoes held at 20 and 26°C were intermediate for both infection and dissemination rates. The effect of environmental temperature should be considered when evaluating the vector competence of these mosquitoes and modeling risk of WN virus transmission in nature.

KEY WORDS West Nile virus, *Culex pipiens*, environmental temperature

The detection and dispersal of West Nile (WN) virus on the eastern seaboard of the United States has heightened interest in the potential for indigenous mosquitoes to transmit this virus. A member of the Japanese encephalitis virus serogroup, family Flaviviridae, WN virus has the potential to cause febrile illness, encephalitis, and occasionally death in humans (Hayes 1989). Although enzootic in Africa, southern/central Europe, and Asia (east to the Indian highlands), this was the first confirmed introduction of this virus into the New World. Before its appearance in North America, WN virus had been isolated from many mosquito species. Most isolates, however, were from *Culex* (*Culex*) spp. mosquitoes (Hayes 1989, Hubalek and Halouzka 1999).

Laboratory studies of North American mosquitoes to evaluate their ability to transmit WN virus (Turell et al. 2000, 2001) indicate that there is a variation in vector competence among these species. However, studies by Turell et al. (2000, 2001) were conducted at a constant temperature of 26°C. Environmental temperature can affect infection, dissemination, and transmission rates for numerous arboviruses, including WN virus (Takahashi 1976, Turell et al. 1985, Watts et al. 1987, Hardy 1989, Turell 1989). Cornel et al. (1993) demonstrated that above normal environmental temperatures shortened the extrinsic incubation period for WV virus in *Culex quinquefasciatus* Theobald. Although most isolations of WN virus from field-collected mosquitoes in North America were made from *Culex pipiens* L. (CDC 2000), this species is only a moderately efficient laboratory vector at 26°C (Turell et al. 2000, 2001). In contrast, *Aedes albopictus* (Skuse), *Ochlerotatus atropalpus* (Coquillett), and *Ochlerotatus japonicus* (Theobald) were significantly more efficient laboratory vectors of WN virus (Turell et al. 2001). These differences in vector competence might be explained by the effect of environmental temperature.

We, therefore, evaluated the effect of temperature during extrinsic incubation on the transmission of WN virus in *Cx. pipiens* to determine if slightly warmer environmental temperatures, consistent with those observed in New York in 1999, increased the vector competence of this species for WN virus.
was recovered from its body, but not its legs, the frozen at -70°C until examined for WN virus. If virus was recovered from both the body and leg suspension, the mosquito was considered to have a disseminated infection (Turell et al. 1984). Previous studies indicate that virtually all Cx. pipiens (Turell et al. 2000, 2001) and similar Culex (Culex) spp. (Sardelis et al. 2001; M.J.T., unpublished data) with a disseminated infection transmit this virus by bite. Thus, we considered that the dissemination rate would be similar to the transmission rate for Cx. pipiens in this study.

### Results

Viremias in the chickens at the time of mosquito feeding ranged from 10^6.6 to 10^7.3 PFU of virus per milliliter of blood. For mosquitoes held at each temperature, infection and dissemination rates were similar for each of the seven viremic feedings. Therefore, data were combined for analysis.

At each of the time intervals sampled after the infectious blood meal, infection rates were consistently lower in mosquitoes held at 18°C than in those held at any of the warmer temperatures, and infection rates increased with increasing holding temperature (Table 1). For the totals of all mosquitoes, the infection rate in mosquitoes held at 15°C was significantly lower (χ^2 = 5.1, df = 1, P < 0.02) than those for mosquitoes held at other temperatures. Likewise, at any given day of extrinsic incubation, dissemination rates were higher in mosquitoes held at higher temperatures. For mosquitoes held at each of the four temperature regimens these rates increased concurrently with extrinsic incubation time (Fig. 1). Although dissemination rates were >50% for mosquitoes held at 30°C by 6 d after the infectious blood meal, dissemination rates never reached 30%, even after 32 d, in mosquitoes that were held at 15°C. Results for the mosquitoes held at 20 or 26°C were intermediate for disseminated infection.

Viral titers in mosquitoes held at all four temperature regimens increased concurrently with extrinsic incubation time. Likewise, at each time interval, average viral titers were directly related to environmental temperature (Fig. 2).

### Discussion

As reported in previous studies using a variety of mosquito-virus combinations (LaMotte 1963, Hurlbut 1973, Jupp 1974, Takahashi 1976, Turell and Lundstrom 1990, Cornel et al. 1993, Brubaker and Turell...
1998, Dohm and Turell 2001), our study indicated that Cx. pipiens maintained at warmer extrinsic incubation temperatures were more likely to become infected with WN virus, and infections became disseminated more rapidly than in conspecific mosquitoes maintained at cooler temperatures. This could help explain the explosive transmission of this virus (62 cases, seven deaths) in New York during the summer of 1999 (CDC 1999b), a year of near record high temperatures (NOAA 2001). In contrast, 21 cases and two deaths were recorded in 2000 (CDC 2001), a year of below average summer temperatures (NOAA 2001). Similarly, above average temperatures were associated with the recent WN outbreaks in Volgograd, Russia, in 1999 (Platonov et al. 2001) and Bucharest, Romania, in 1996 (Han et al. 1999, Savage et al. 1999). Additionally, Hess et al. (1963) theorized that unusually high temperatures contributed to epizootics of St. Louis encephalitis (SLE) virus in the United States.

Studies by Turell et al. (2000, 2001) indicate that ~90% of Cx. pipiens with a disseminated infection transmit WN virus by bite. Thus, nearly all Cx. pipiens with a disseminated infection in this study would be expected to be able to transmit WN virus. The accelerated occurrence of disseminated infections in mosquitoes held at 30°C in our study (Fig. 1) indicates that these mosquitoes could transmit WN virus efficiently at their first feeding attempt after ingesting an infectious bloodmeal. In contrast, Cx. pipiens exposed to lower environmental temperatures would take longer to develop a disseminated infection and would be correspondingly less efficient transmitters of WN virus.

The relationship between environmental temperature and the ability of mosquitoes to transmit an ar-
bovirus is not consistent. Although most studies have found a direct relationship between temperature and both infection and dissemination rates, Kramer et al. (1983) found that Culex tarsalis Coquillett was a more efficient vector of western equine encephalomyelitis (WEE) virus when held at cooler, rather than warmer, temperatures. This is consistent with a study by Hess et al. (1963) that found that outbreaks of WEE were more common in years with below average temperature. Our results indicate that in Cx. pipiens exposed to WN virus the relationship between environmental temperature is of the more typical pattern (i.e., mosquitoes held at higher temperatures were more efficient vectors).

Various factors in addition to vector competence determine the potential for virus transmission. These include population density, feeding behavior, and flight range of potential vectors; population density of suitable amplification vertebrate hosts; presence of hosts susceptible to disease; and relative virulence of the virus. These must be combined with anti-mosquito control activities, education and use of personal protective measures, and various environmental factors (e.g., rainfall and temperature) to determine the risk of a potential disease outbreak.

Based on the reduced extrinsic incubation period for WN virus in Cx. pipiens held at higher temperatures in our study, similar results from other studies, and environmental conditions observed during the recent WN outbreaks in New York, Russia, and Romania, warmer than average seasonal temperatures could promote epizootics of this virus. The distribution of WN virus continues to expand, and WN activity was detected in 12 states and the District of Columbia in 2000, as opposed to four states in 1999 (CDC 2000). Thus, as WN virus extends its distribution into the southern United States, and possibly Central and South America, its transmission is likely to be even more intense due to the warmer climate.

Acknowledgments

We thank T. McNamara (Wildlife Conservation Society) for providing the Crow 397-99 strain of WN virus and J. Oliver (Arthropod-borne Disease Program, New York State Department of Health, Syracuse, NY) for providing the Cx. pipiens used in this study. We also thank D. Schachner and Cornel, A. J., P. G. Jupp, and N. K. Blackburn. 1993. Environmental temperature on the vector competence of Culex quinquefasciatus (Diptera: Culicidae) for West Nile virus. J. Med. Entomol. 30: 449–450.

References Cited


Received for publication 24 May 2001; accepted 2 October 2001.