Mosquito-borne West Nile virus (WNV) surveillance in the Upper Rhine Valley, Germany

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ABSTRACT: West Nile virus (WNV) could be introduced into Germany via migratory birds originating from Africa or southern Europe and subsequently transmitted to indigenous birds, humans, or horses by mosquitoes. Neither the virus itself nor antibodies against WNV have yet to be found in mosquitoes and horses, whereas antibodies have been detected in migrating birds and in humans that were in close contact with birds. At present, the West Nile virus itself has yet to be detected in Germany. This investigation was conducted primarily in major bird breeding, resting, and roosting habitats (hotspots) in the Upper Rhine Valley. Adult mosquitoes were trapped using CO_2-baited Encephalitis Vector Surveillance (EVS)-traps and were tested for WNV by the VecTest WNV Antigen Assay. In 2007 and 2008, a total of 11,073 host-seeking adult female mosquitoes (13 species) were tested, and all tests were negative for WNV. Statistical calculations could be performed only where sufficient numbers of mosquitoes were trapped. For these sites, WNV infection among mosquitoes could be ruled out with 80% certainty. For the evaluation of the WNV situation in Germany, the results of this investigation are a further indication that the virus has not yet arrived. ***Journal of Vector Ecology* 35 (1): 140-143. 2010.**

Keyword Index: West Nile virus, arbovirus, mosquitoes, surveillance, *Aedes*, Germany.

INTRODUCTION

Mosquitoes are the primary vectors of West Nile virus (WNV; Family Flaviviridae), while birds serve as amplifying hosts, and humans and some mammals are dead-end hosts. WNV was identified for the first time in 1937 in Uganda (Africa), and in some parts of Europe the virus is deemed to be endemic. WNV cases have been reported from 20 European countries (Hubálek and Halouzka 1999, Becker et al. 2003). Since 1996, the number of severe cases (with neurological symptoms) of WNV has increased (Stock 2004) and since 1999, the virus has been spreading rapidly throughout the U.S.A.

Although WNV infections in Germany have yet to be detected in humans, individuals that had close contact with birds (i.e., bird banders) tested seropositive. These individuals displayed no WNV symptoms but had been traveling in countries with recorded WNV occurrence. No antibodies were found in horses or in humans showing West Nile fever-like symptoms. Migrating birds overwintering in Africa or southern Europe and captured in Germany (10%) tested positive for WNV antibodies (Linke et al. 2007, 2008).

WNV has been found in 75 mosquito species worldwide. Nine of these species occur in the Upper Rhine Valley: *Culex pipiens* Linnaeus, *Cx. territans* Howard, Dyar & Knab, *Culiseta morsitans* Theobald, *Aedes vexans* Meigen, *Ae. cinereus* Meigen, *Ae. sticticus* Meigen, *Ae. excrucians* Walker, *Anopheles maculipennis* Meigen, and *Coquillettidia richiardii* Ficalbi. An isolated occurrence of *Ae. albopictus* Skuse was recorded in 2007 (Pluskota et al. 2008).

Apart from mosquito vector competence, two factors have considerable impact on its role in transmitting WNV: the first factor is feeding preferences. Ornithophilic species can potentially spread the virus within the bird population, whereas species that feed on both birds and mammals can serve as bridge vectors. The second factor is the density of a mosquito species. In this context, the temporary mass occurrence of flood-plain mosquitoes in the Upper Rhine Valley (primarily *Ae. vexans*) is noteworthy. This species preferentially feeds not only on humans, but also on birds; therefore, it could potentially serve as a bridge vector (Turell et al. 2005), as well as an enzootic vector (Tiawsirisup et al. 2008).

Since 2003, mosquitoes in the Upper Rhine Valley have been tested to identify the presence of WNV. Between 2003 and 2006, a total of 837 larval and adult *Culex* specimens were tested (unpublished data). In 2007 and 2008, testing was extended to include additional mosquito species, the results of which are presented here.

MATERIALS AND METHODS

Times and areas of investigation

It was not the intention of this investigation to compare the mosquito population of different years or areas, but to trap as many mosquitoes as possible for WNV testing within a short time. The areas were chosen because of their importance as bird hubs in combination with their high contingent of mosquitoes with ornithophilic feeding preferences or their total density of mosquitoes. According to Gu et al. (2008), an increased sampling effort at a few

hotspots ("targeted surveillance") is more suitable for early arbovirus detection than sampling a large area with less effort at each site ("random surveillance"). The probability of detecting WNV infection in a mosquito population increases with the infection rate of the mosquitoes. During the "mosquito-season" (March through September), the infection rate in mosquitoes can increase both due to the viral replication and dissemination within infected mosquitoes and transmission from vertebrate hosts. In order to increase the probability of detecting the virus, adult mosquitoes were trapped in August and early September, mainly in major bird breeding and resting habitats.

All study areas were situated in the Upper Rhine Valley. The Wagbachniederung (investigated in 2007 and 2008) and Kühkopf-Knoblochsaue (2008) Nature Reserves were chosen because of their importance as hubs for migrating birds. In 2007, mosquitoes from one hotspot and nine routine monitoring sites, located within the boundary of the German Mosquito Control Association (KABS), were tested (Nonnenaue, Junge Gründe, Honau, Leutesheim, Greffern 1-4, Kulturwehr). In 2008 the strategy was changed to sampling only hotspots in order to improve efficiency (Gu et al. 2008).

Encephalitis virus surveillance (EVS) traps

We used EVS traps with dry ice (CO_2) as a bait to attract host-seeking female mosquitoes. An insulated dry ice container with small holes for sublimation of the CO_2 was placed over the trap. Mosquitoes attracted to CO_2 bait are trapped in the net attached to the trap. Depending on the mosquito density of the respective area, up to 16 traps were set per night in the areas investigated at ~1.5 m height within the vegetation. In the morning, mosquitoes were killed by freezing and transported to the laboratory for species identification (Becker et al. 2003) and WNV testing.

Table 1. Mosquito numbers and species tested for WNV in 2007 and 2008.

Species	2007	2008	Total	%
Culex pipiens/torrentium	115	93	208	1.88
Culex modestus	5	6	11	0.10
Aedes vexans	3,653	3,929	7,582	68.47
Aedes cinereus	332	97	429	3.87
Aedes rossicus	518	2	520	4.70
Aedes sticticus	1,267	443	1,710	15.44
Aedes annulipes	5	3	8	0.07
Aedes geniculatus	10		10	0.09
Culiseta annulata	97	52	149	1.35
Anopheles maculipennis	35	30	65	0.59
Anopheles claviger	176	176	352	3.18
Anopheles plumbeus	13	6	19	0.17
Coquillettidia richiardii	3	7	10	0.09
Total	6,229	4,844	11,073	

VecTest® WNV antigen assay

The VecTest® (Microgenics Inc., Freemont, CA, U.S.A.) assay was performed according to manufacturer's instructions. Mosquitoes were tested immediately and, in one case, in a dried condition. The mosquitoes were tested in pools up to 50. They were homogenized for 1 min at high speed and 250 µl of the homogenate was dispensed into the conical tube provided and a test strip inserted. After waiting for at least 15 min, a minimum of two observers read the test results.

RESULTS

In 2007 and 2008, a total of 11,073 adult female mosquitoes from 13 species was tested. All mosquito pools were WNV-negative (Table 1).

DISCUSSION

WNV vectors in the Upper Rhine Valley

The mosquito species identified in the present study are potential vectors of WNV. Biological and ecological aspects of virus vectors and hosts, such as feeding preferences and density of both, may restrict virus transmission. Mosquito species identified as primary vectors of WNV have been found in different parts of the world, e.g., *Cx. pipiens* (Turell et al. 2000, Andreadis et al. 2004) and *Cx. pipiens quinquefasciatus* (Vanlandingham et al. 2007) in the U.S.A., *Mansonia uniformis* in Bulgaria, *Ae. cinereus*, *Ae. vexans,* and *Cx. pipiens* in the Czech Republic, *Cx. modestus* and *Cx. pipiens* in France, *An. maculipennis* in Portugal, *Ae. cantans* in Slovakia, *Cx. pipiens* in Romania, *Ae. vexans*, *Cx. modestus,* and *Cx. univittatus* in the Russian Federation, *An. maculipennis* in the Ukraine, and *Cx. perexiguus* in Israel (Orshan et al. 2008).

Culex pipiens has been regularly identified as a vector of WNV. Therefore, areas with a high density of *Culex pipiens* have been primarily chosen for this investigation. Because of its ornithophilic feeding preferences, this species is able to spread the virus effectively within a bird population, but it is not very effective in its ability to transmit it to humans. Therefore, both the infection rate and the mosquito population must be very high to lead to WNV transmission to humans (Gu et al. 2006). What species were to be the primary vector if WNV were to be introduced into Germany cannot be known yet, but assumptions can be made due to the biology and ecology of the abundant species.

In the Upper Rhine Valley, 34 mosquito species have been found (Becker and Kaiser 1995, Pluskota et al. 2008). The main nuisance mosquito is *Ae. vexans*, which is regularly found as >80% of the total mosquito numbers caught in the KABS monitoring program. This species is a floodplain mosquito with high density during summer floods. It feeds mainly on mammals and occasionally on birds (ca. 7%, Molaei and Andreadis 2006). Humans are among the preferred hosts of *Ae. vexans*; therefore, it is

presumably a suitable bridge vector for WNV in some areas (Turell et al. 2005). Vector competence in *Ae. vexans* was studied in several investigations and it was determined to be a competent vector for WNV in several countries (Bernard et al. 2001, Andreadis et al. 2004, Anderson et al. 2006, Tiawsirisup et al. 2008).

In the laboratory, it was demonstrated that in addition to birds, mammals (e.g., cottontail rabbits, squirrels, chipmunks) can develop WNV titers that are infectious to *Ae. vexans*; therefore, this species could serve as an enzootic vector. Moreover, the vector competence of *Ae. vexans* may be similar to that of *Cx. pipiens*. However, in different *Ae. vexans* populations, varying degrees of vector competence were recorded (Tiawsirisup et al. 2008, Platt et al. 2007). In the U.S.A., WNV has been detected every year since 1999 in *Ae. vexans* and *Cx. pipiens*.

Incidence of WNV-infected mosquitoes

The lack of WNV detection among mosquitoes in the present study does not necessarily signify a lack of WNV circulation in the Upper Rhine Valley. In some of the investigated areas (see below), the number of mosquitoes was high enough for a statistical calculation of the probability of WNV infections. According to Gu and Novak (2004), a sample size of >1,600 mosquitoes is necessary to exclude an infection significantly, assuming an 80% probability of detection and an infection rate of 1:1,000. But this minimum is only valid with 100% sensitivity of the testing method. Various researchers observed that using the VecTest, the number of infections detected was less than the actual number of infections (Burkhalter et al. 2006, Lampman et al. 2006, Nasci et al. 2002). The lowest percentage of detection with VecTest found in these investigations was 60%. Therefore, an increase of the sample size to 2,240 (plus ~40%) mosquitoes seems to be sufficient. A precondition for this method of calculation is that the tested mosquitoes be trapped in the same habitat within a short period of time. An aggregation of several samplings over a longer period or of different areas is not adequate. Under these assumptions and based on statistical calculations according to Gu and Novak (2004), WNV infection of the mosquito population can be significantly excluded with a probability of 80% in some areas (Wagbachniederung and Nonnenaue in 2007 as well as for Kühkopf-Knoblochsaue in 2008). In the other investigated areas (see above), the number of mosquitoes caught in the EVS traps was not high enough for these statistical calculations.

At present, the occurrence of WNV in Germany appears to be unlikely, because all tests on birds, humans, horses, and mosquitoes have been negative. With the possible arrival of the pathogen in Germany, *Ae. vexans* could be considered a potential bridge vector in the Upper Rhine Valley for transmission to humans. This species is a competent vector for WNV; it bites mammals, humans, and also feeds on birds when it occurs in large numbers.

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