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Lack of Evidence for the Presence of Mosquito-Borne Arboviruses in the Upper Rhine Valley, Germany, in 1999 to 2000[▽]

Several mosquito-borne arboviruses are known in Europe, but only Tahyna virus (*Bunyaviridae*, *Orthobunyavirus*) has been isolated in Germany (2, 6, 11). Recently, in 2009, Sindbis virus has also been detected and isolated from 16,057 mosquitoes sampled in Germany (10 Sindbis virus RNA positives, 3 Sindbis virus strains) (3). In two previous years, we have tested a very large number of mosquitoes, sampled in the same area of Germany, without detecting any Sindbis virus activity. Therefore, it is interesting and important to contrast the results of these two studies.

In 1999, 13 mosquito species of five genera were captured and investigated for virus by inoculation of Vero cells (Table 1). No cytopathogenic agent was found in the 59,915 mosquitoes tested in 1,342 pools. Sindbis virus is usually most prevalent in *Culex* mosquitoes (1); it has not been isolated from *Aedes vexans* in the field (7), and this prevalent species has a midgut infection barrier to Sindbis virus (8). Thus, to increase the possibility of detecting Sindbis virus activity, the strategy was to focus on *Culex* species mosquitoes in the following year. In 2000, the study was performed from 20 June to 7 September in Bobenheim-Roxheim, 22 km from Weinheim, Germany, and a total of 24,525 *Culex* mosquitoes were collected and assayed for virus. However, no cytopathogenic agent was found in the 995 pools tested.

The different findings between the 1999–2000 study (84,440 mosquitoes, no virus detected) and the 2009 study raise interesting questions about annual variation in Sindbis virus activity, introduction of virus versus low-level endemicity, and the selection of methods and strategies for detecting virus activity in mosquitoes.

TABLE 1. Mosquito species composition and numbers of females per species caught in the Upper Rhine Valley in 1999 and in Bobenheim-Roxheim in 2000 and investigated by virus isolation in Vero cells[a]

Species	No. of individuals (no. of pools)[b]	
	1999	2000
Aedes vexans	32,431 (678)	
Aedes sticticus	9,177 (209)	
Aedes cinereus	2,078 (58)	
Aedes rusticus	116 (8)	
Aedes annulipes	108 (7)	
Culex pipiens/Culex torrentium[c]	10,101 (217)	4,306 (139)
Culex modestus	3,492 (73)	20,219 (856)
Culiseta annulata	1,038 (40)	
Coquillettidia richardii	110 (5)	
Anopheles maculipennis	1,161 (41)	
Anopheles claviger	66 (3)	
Anopheles plumbeus	37 (3)	
Total	59,915 (1,342)	24,525 (995)

[a] No virus isolates were obtained. The pool size never exceeded 50 individuals, with its composition following the strict hierarchic roles to never mix mosquito species, never mix trapping sites, and to mix trapping dates if necessary but not when more than 1 week apart.

[b] The total number of mosquitoes caught and the total number of pools in 1999 and 2000 were 84,440 and 2,437, respectively.

[c] Sibling species *Culex pipiens* and *Culex torrentium* were not tested separately because adult females cannot be distinguished morphologically.

One well known but poorly understood phenomenon in the epidemiology of Sindbis virus infections in Scandinavia is its cyclic peak occurrence every seventh year since its first description in Sweden in 1967. Clusters of human cases were described in Finland in 1974, 1981, 1988, 1995 (>1,300 cases), and 2002 (597 cases) (4). Although far-fetched, the isolation of Sindbis virus in Germany in 2009 (epidemic year) fits into this 7-year cycle and would explain why no isolates were obtained in the years 1999 (nonepidemic) and 2000 (nonepidemic). A recent Sindbis virus introduction and several years of low-level virus activity are difficult to differentiate. The mechanism for long-range geographic spread of Sindbis virus is not known in detail (7, 9). Migrating birds are often suggested as responsible for long-distance dispersal of mosquito-borne arboviruses, but data supporting this are sparse (10).

Apparently, the real-time reverse transcription (RT)-PCR for detection of Sindbis virus-specific RNA is more sensitive than the method of virus isolation on Vero cells, since these methods provided 10 RNA positives and 3 virus isolates, respectively, from the same mosquito material (3). Since Jöst et al. (3) did not provide information on the mosquito species composition and the numbers of mosquitoes and pools tested per species, it is difficult to compare the results between the two studies. Three Sindbis virus isolates from 4,016 *Culex* mosquitoes collected in Weinheim in 2009 versus no virus isolates from 13,593 *Culex* mosquitoes (1999) or from 24,525 *Culex* mosquitoes (2000) collected in nearby Bobenheim-Roxheim might simply indicate a higher Sindbis virus activity in *Culex* mosquitoes during 2009. However, without knowledge of the number of *Culex torrentium* mosquitoes, the main enzootic vector for Sindbis virus (5) tested for virus, it will be difficult to discern any differences in mosquito infection rates between years and between geographic areas.

We can rule out neither that Sindbis virus had been present in the upper Rhine Valley for a long time before 2009 nor that the virus had been recently introduced. However, we like to emphasize that further studies are needed to understand the transmission dynamics and the pathogenic potential of Sindbis virus in Germany. Serology should be used to investigate the eventual occurrence of Sindbis virus-specific antibodies in humans and birds, for investigation of human cases resembling the clinical picture of a Sindbis virus infection, and to determine the pathogenic potential of the particular virus strains found.

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