

First record of mosquito-borne Sindbis virus (genotype I) in the Czech Republic

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Received January 28, 2022; revised April 19, 2022; accepted May 4, 2022

Keywords: Sindbis; Culex; mosquito; Central Europe

Due to globalization and climate change, mosquito-borne pathogens pose a significant health threat to humans. Several mosquito-borne viruses circulate in Central Europe, of which West Nile virus (WNV) currently poses the greatest epidemiological risk. Other arboviruses circulating in Central Europe with lesser medical impact include Tahyňa virus (TAHV), Čalovo virus (CVOV), Usutu virus (USUV), and Sindbis virus (SINV) (1). The disease caused by SINV is called Sindbis fever (Karelian/Ockelbo/Pogosta fever in Fennoscandia) and is characterized by sudden onset of headache and arthralgia or arthritis, rash on the thorax and extremities; as these clinical signs are also observed with other pathogenic alphaviruses (2). Convalescence can be quite protracted (joints may ache for months or even years), arthralgia, arthritis, and rheumatic symptoms are observed in nearly 25% of patients for up to 3 years after the acute illness (1,3). In Central Europe, there is little evidence that SINV is spread by mosquitoes, birds, or humans, and the disease remains unrecognized in humans and misdiagnosed as a fever of unknown etiology. Historically, indirect detection of SINV was first documented in the former Czechoslovakia in 1971, when antibodies to SINV were detected in birds (4). Shortly thereafter, SINV was isolated from the blood and liver of a reed warbler (*Acrocephalus scirpaceus*) in western Slovakia (5) and at the same time the virus was isolated from the blood and liver of sentinel hamsters

in eastern Slovakia (6). The continuous spread of SINV was documented in 1973, when six strains of SINV were isolated from sentinel ducks in western Slovakia, particularly in the Malacky region (7), and later from the frog *Rana ridibunda* (8). In the Czech Republic, antibodies to SINV were found only rarely in southern Moravia and southern Bohemia (9). In southern Moravia, 106 wild geese (*Anser anser*) were tested for virus-neutralizing antibodies to SINV; 14.2% were positive (10). Two hundred and ninety-five passerine birds captured in southern Moravia were analyzed by Juřicová *et al.* (11) using the hemagglutination inhibition test (HIT), and 6.4% of them revealed SINV antibodies. In the same area, 178 passerine were tested later, only 0.7% were seropositive by HIT (12). Antibodies to SINV were detected in 2.9% of birds from the *Hirundinidae* family (n=183) by HIT (13). Of 31 cormorants (*Phalacrocorax carbo*) in southern Moravia, 9.7% had HI antibodies to SINV (14). Juřicová *et al.* also tested by HIT 273 house sparrows (*Passer domesticus*) captured in northern Moravia, and SINV antibodies were detected in 2.2% of them (15). Of 93 wild boars examined serologically (HIT) in southern Moravia, only one was positive (16). Kolman (17) found no seropositivity to SINV by HIT in domestic mammals (61 horses, 305 cows, and 109 pigs) in southern Moravia (Břeclav district), but antibodies were present in 1.0% of 104 hens and 8.5% of 47 ducks. Of 574 domestic ducks kept on five fish ponds in southern Moravia, only 0.3% were seropositive by HIT (18). Interestingly, the main vector of SINV, the mosquito *Culex pipiens*, has never been detected in the Czech Republic and Slovakia. To expand our knowledge of the presence of pathogenic SINV in local mosquito populations, we conducted massive arbovirus

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Abbreviations: HI(T) = hemagglutination inhibition (test); SINV = Sindbis virus; WNV = West Nile virus



Fig. 1

Phylogram demonstrating relationship of Sindbis virus (genotype I) detected in mosquitoes in the Czech Republic and other Sindbis virus strains circulating worldwide

Each record consists of particular accession number, source (human/mosquito/bird), place and year of detection/isolation. Czech samples are highlighted by red triangles. Phylogenetic analyses were conducted using Maximum Likelihood (ML) algorithm (MEGA 7.0) using the General Time Reversible model + G + I (G parameter = 0.7682, I = 0.4454). Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree with the highest log likelihood (-3081.88) is shown. The robustness of trees was tested by bootstrap resampling of 1000 replicates and its values are listed near the nodes (only values ≥ 90 are shown). The horizontal bar shows genetic distance. (Legend: BABV - Babanki virus (SINV subtype); OCKV - Ockelbo virus (SINV subtype); SINV - Sindbis virus; WEEV - Western equine encephalitis virus-outgroup).

surveillance at local fish ponds, where the putative SINV mosquito vectors *Cx. pipiens* and *Cx. modestus* co-occur with wild waterfowl, the primary reservoir hosts of SINV (19). In this study, a total of 10,235 *Cx. pipiens* and *Cx. modestus* mosquitoes were captured in 2019 and 2020 as part of several large-scale surveillance projects in an area endemic to WNV. A detailed description of mosquito habitat, capture, identification, and storage, can be found in Sikutova *et al.* (20). Monospecific pools of 50 females of *Cx. modestus* or *Cx. pipiens* were prepared in the laboratory. They were homogenized in 1.5–2.0 ml of chilled phosphate-buffered saline pH 7.4 supplemented with 0.4% bovine serum albumin fraction V (Sigma), penicillin (500 IU/ml), streptomycin (100 μ g/ml) and gentamicin (100 μ g/ml). The homogenates were centrifuged at 1,500 g for 20 min (at 0°C) and the supernatant was used for RNA extraction. RNA was extracted from 140 μ l of the mosquito homogenates using the QIAamp viral RNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. All suspensions were tested for alphaviruses by broad reverse transcriptase-polymerase chain reaction (RT-PCR) using generic primers designated 'VIR966' (21). To obtain a more representative genome segment for phylogenetic reconstruction, we then tested alphavirus-positive pools by amplifying a subregion (764 bp) of SINV with the primers SIN 15F and SIN 15R, specifically positions 8,122 to 8,885 of the SINV polyprotein gene encompassing the E2 region (20, 22). A total of 3,546 *Cx. pipiens* individuals in 76 pools and 6,689 *Cx. modestus* individuals in 138 pools were examined for the presence of pathogenic alphaviruses. Two *Cx. modestus* pools (designated '19-93' and '20-67'), geographically located at the reservoir 'Nove mlyny' (sampled on August 7, 2019) and the fish pond 'Lednicke rybníky' (sampled on August 26, 2020), respectively, proved positive for SINV RNA. Subsequent sequence analysis revealed the presence of Sindbis virus (genotype I). The Czech sequences were deposited in the GenBank database under Acc. Nos. OL456242 and OL456243, respectively. Both strains have 99% sequence similarity to two Russian SINV strains designated 'Tatarstan' (MG679380) and 'LEIV-Ast03-1-844' (MG679378) and the SINV strain 'F-720' (MG679376) isolated from *Bubulcus ibis* in Armenia. Sequencing of other parts of the genome failed because of the insufficient amount of starting material (mosquito homogenates were previously used to screen other arboviruses). In conclusion, we report the first detection of mosquito-borne Sindbis virus (genotype I) on the territory of the Czech Republic. Despite massive surveillance efforts focused on mosquito-borne viruses in the former Czechoslovakia and then in large-scale European projects, the SINV vector remained unnoticed for many years. In contrast to Central Europe, SINV is the most important arbovirus in Fennoscandia and causes large epidemics in

humans with a periodicity of 7 years that coincides with the population cycles of tetraonid birds (23). Migratory birds can transmit this virus over long distances. Therefore, surveillance efforts should focus on reed beds with high concentrations of birds and bird-feeding mosquitoes, especially *Cx. modestus* species. Recently, RNA of the Kyzylgach variant of SINV (genotype IV) was detected by PCR in four of 221 pools prepared from 10,784 female *Culex modestus* mosquitoes collected at a fishpond in southern Moravia (20) – the first time in Central Europe. From an epidemiological perspective, only a “One Health” approach combining the expertise of entomologists, microbiologists, infectious disease specialists and epidemiologists, can effectively assess the true impact of SINV exposure in Central Europe.

Acknowledgments. The study was financially supported by the Ministry of Health of the Czech Republic (Reg. No. NV19-09-00036).

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