Mosquitoborne Sindbis Virus Infection and Long-Term Illness

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DOI: https://doi.org/10.3201/eid2406.170892

An unexpected human outbreak of the mosquitoborne Sindbis virus occurred in a previously nonendemic area of Sweden. At follow-up, 6–8 months after infection, 39% of patients had chronic arthralgia that affected their daily activities. Vectorborne infections may disseminate rapidly into new areas and cause acute and chronic disease.

Mosquitoborne viruses such as chikungunya virus, Ross River virus, and Sindbis virus (SINV) are members of the genus *Alphavirus* (family *Togaviridae*) and cause human arthritic diseases (*1*). SINV has mainly been reported in northern Europe and South Africa (*1*); Sweden has an average of 3 SINV cases per year, with occasionally more cases in a previously defined endemic region in central Sweden (Figure) (*2*). Birds are the reservoir for SINV, and there is no evidence of human-to-human transmission. SINV infection in humans, called Ockelbo disease in Sweden, causes rash, arthritis, and mild fever (*3–5*). Most patients recover within weeks or months, but arthralgia and myalgia can persist for years following infection, suggesting inflammatory response or a persistent infection (*4–6*).

In mid-August 2013, several patients with rash, arthralgia, and fever visited the healthcare center in the small village of Lövånger in Västerbotten County, Sweden (Figure). The university hospital laboratory in Umeå received 172 blood samples from patients with suspected SINV infection; 50 patients had SINV-specific IgM and IgG (online Technical Appendix, https://wwwnc.cdc.gov/EID/article/24/6/17-0892-Techapp1.pdf). SINV infections have been believed to be almost exclusively confined to the central part of Sweden (*2*), but the 2013 outbreak occurred north of the endemic area (Figure). The distribution of verified cases by sex in this outbreak showed a higher proportion of female patients (62%) than male patients (38%) with acute SINV infection (online Technical Appendix Table 1).

Previous reports suggested that joint symptoms might persist for years in SINV infections (*4–6*). To evaluate long-term consequences, we contacted 46 SINV patients by telephone 3–4 months, 6–8 months, or in both periods after acute disease (online Technical Appendix Figure 1). Our study was approved by the Regional Ethics Review Board (2014-102-32M), and we obtained written informed consent from all participants. We include details of the study results summarized here in the online Technical Appendix. In total, 18/46 (39%) of the patients reported persistent musculoskeletal pain (arthralgia and myalgia) and restriction in their daily activity 6–8 months after the onset of acute symptoms (online Technical Appendix Table 2). We invited these patients for a standardized examination by a rheumatologist, including a health assessment

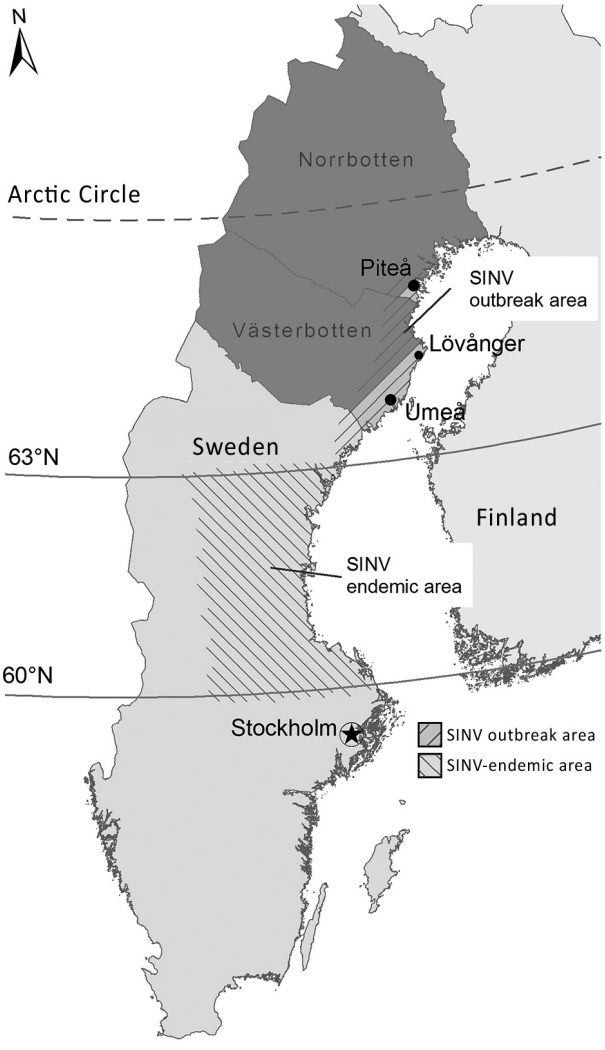

Figure. Geographic distribution of the SINV outbreak in 2013 and previous occurrence of SINV infections in Sweden. Dark gray indicates the 2 northernmost counties in Sweden where the SINV IgG seroprevalence was 2.9% in 2009. SINV, Sindbis virus.

questionnaire, patient assessments of their pain and their global health using a visual analog scale, and a blood sample (online Technical Appendix). Of 17 symptomatic patients who participated, 1 had arthritis in the ankle, 14 had ≥1 tender joint, and 10 had enthesitis, tendinitis, or tenosynovitis at examination. Large joints (knee, hip, shoulder, wrists, ankles) and small joints (toes, fingers) were affected, with a predominance for the lower extremities, in contrast to other studies in which small and peripheral joints were mainly affected (*4*–*6*). Patients graded their global symptoms as more severe than the examining doctor did, indicating that joint function was only mildly affected whereas the pain was perceived as restricting. Test results did not detect citrulline antibodies, and the single patient with positive rheuma factor had no arthritic symptoms. A notable finding was that 4 patients (24%) had psoriasis, a condition present in <4% of the northern European population (*7*), raising the question whether psoriasis makes SINV patients more vulnerable to long-term arthralgia.

We asked the 28 patients at the 6- to 8-month follow-up who had recovered to complete a questionnaire and donate a blood sample at their local healthcare provider; 23 did so. Symptomatic patients reported more pain and impaired health, compared with patients who were asymptomatic 6–8 months after acute disease. We detected SINV-specific IgM in patients with and without persistent symptoms, as previously reported (*5*).

We isolated a new SINV strain from a mosquito caught in the area during the outbreak; it was most closely related to a SINV strain from Finland (*8*). However, no increased incidence was recorded in Finland either the year before or concomitantly with the Swedish outbreak (*9*). In addition, only 1 case was reported from the endemic area of central Sweden in 2013, suggesting that local factors such as weather conditions may determine an outbreak. June 2013 stood out with a high mean temperature and precipitation, which have been shown to be associated with a high incidence of SINV infection later in summer (online Technical Appendix Figure 2).

A recent study in northern Sweden revealed that in a randomly selected population-based cohort, 2.9% had SINV IgG (Figure), indicating that the virus was present in the region, although not recognized (*10*). More research is warranted regarding the long-lasting joint pain caused by a previous SINV infection; patients with undiagnosed SINV may visit a healthcare facility with such symptoms even several months postinfection. Our report illustrates how a vectorborne zoonotic disease can result in a large, unexpected outbreak. The key factors for outbreaks of SINV or other alphavirus-caused diseases are generally unknown, which warrants further investigations, especially in light of the global emergence of alphaviruses (*1*).

Acknowledgments

We thank Maj Bylund for technical assistance.

The study was supported by the County Council of Västerbotten, the Swedish Research Council Formas (grant no. 221-2014-1556), the Medical Faculty of Umeå University, and the Arctic Research Center at Umeå University, ARCUM.

About the Author

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Article DOI: https://doi.org/10.3201/eid2406.170892

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Technical Appendix

Case Definition

We selected cases on the basis of the patient samples that were sent to the clinical microbiology laboratory at Norrland University Hospital, Umeå, Sweden for Sindbis virus (SINV) diagnosis during August–October 2013. We analyzed all samples for IgG and IgM SINV antibodies. Case-patients either had IgM and IgG antibodies against SINV in a single serum sample or showed seroconversion in a follow-up sample. The clinicians at local healthcare centers in northern Sweden referred serum samples to the laboratory based on the combined acute symptoms of joint pain, rash, and sometimes fever, muscle pain, or both. The healthcare centers had been alerted by the Västerbotten County Center for Disease Control, who had sent out a request to look for patients with these acute symptoms, because there had been many cases in a short time and it was not known at that time which agent caused the symptoms.

Immunofluorescence Assay

We analyzed the presence of SINV-specific antibodies in the blood samples with an indirect immunofluorescence assay (IFA) (*1*) used in routine diagnostics at Norrlands University hospital, Umeå Sweden. The antigen was SINV Edsbyn 5/82 (*2*) cultured to 60%–70% cytopathogenic effect in Green Monkey Kidney cells in Dulbecco´s Modified Eagle´s Medium (Sigma-Aldritch, St. Louis, MO, USA) supplemented with 2% Fetal Bovine Serum (Sigma-Aldritch). We harvested infected cells with a cell scraper, washed them twice in phosphate buffered saline (PBS) pH 7.4 and spotted to 70% confluence in 6 mm wells on Teflon-coated glass slides. We allowed slides to dry at room temperature overnight, fixed them in acetone, and stored them at −70°C for later use in IFA. We diluted serum samples 1:10 and 1:40 in PBS and incubated them on the glass slides for 1 hour at 37°C. After washing for 10 minutes in PBS, we

incubated the slides for 1 hour at 37°C with polyclonal rabbit anti-human FITC conjugated IgG or IgM (DAKO, Denmark). After rinsing slides with PBS for 10 minutes, we mounted them with glycerol for fluorescence microscope examination to determine specific granular fluorescence. We included a positive control (human serum previously tested positive for SINV-antibodies) in every batch. We verified all serum samples that were reactive in the IFA with a SINV-specific EIA. To compare the performance of the IFA, we analyzed samples by both methods, 172 samples for IgG and 167 samples for IgM analysis. For IgG detection, the IFA had 76.2% sensitivity and 99.1% specificity compared with the EIA. For IgM detection, the IFA had 96.1% sensitivity and 81.7% specificity compared with the EIA.

ELISA

We measured specific IgG against SINV by an enzyme immunoassay (EIA) as previously described (*3*). Briefly, the SINV antigen, we water-sonicated SINV Lövånger 2013 (*4*) on ice 4 times for 30 s each and box-titrated it to determine the optimal antigen dilution. We incubated diluted serum samples (1:420) at 4°C overnight on antigen-coated wells, washed 4 times with PBS-T, again incubated for 60 min at 37°C with 100 µL goat anti-human IgG alkaline phosphate conjugate (Invitrogen Corporation, USA, lot: 722339A) (1:6000). We added substrate (p-nitrophenyl phosphate disodium; Sigma Diagnostics, USA) and incubated for 30 min at 37°C; we recorded the optical density (OD) at 405 nm. We subtracted the mean OD of 8 blank wells (containing PBS-T and 1% milk) from each result. We determined the cutoff in SINV IgG EIA by analyzing 32 serum samples previously confirmed SINV negative by immunofluorescence assay. We preliminarily determined the assay cutoff by counting the mean OD value of these SINV negative samples +3 standard deviations. We analyzed all serum samples once and those with an OD above cutoff were analyzed again in duplicate. The SINV antibody-positive results from the EIA had previously been verified using a SINV-specific haemagglutination inhibition (HI) test (*3*,*5*).

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Technical Appendix Table 1. Laboratory diagnosis Sindbis virus in patients, Sweden, August–October 2013*

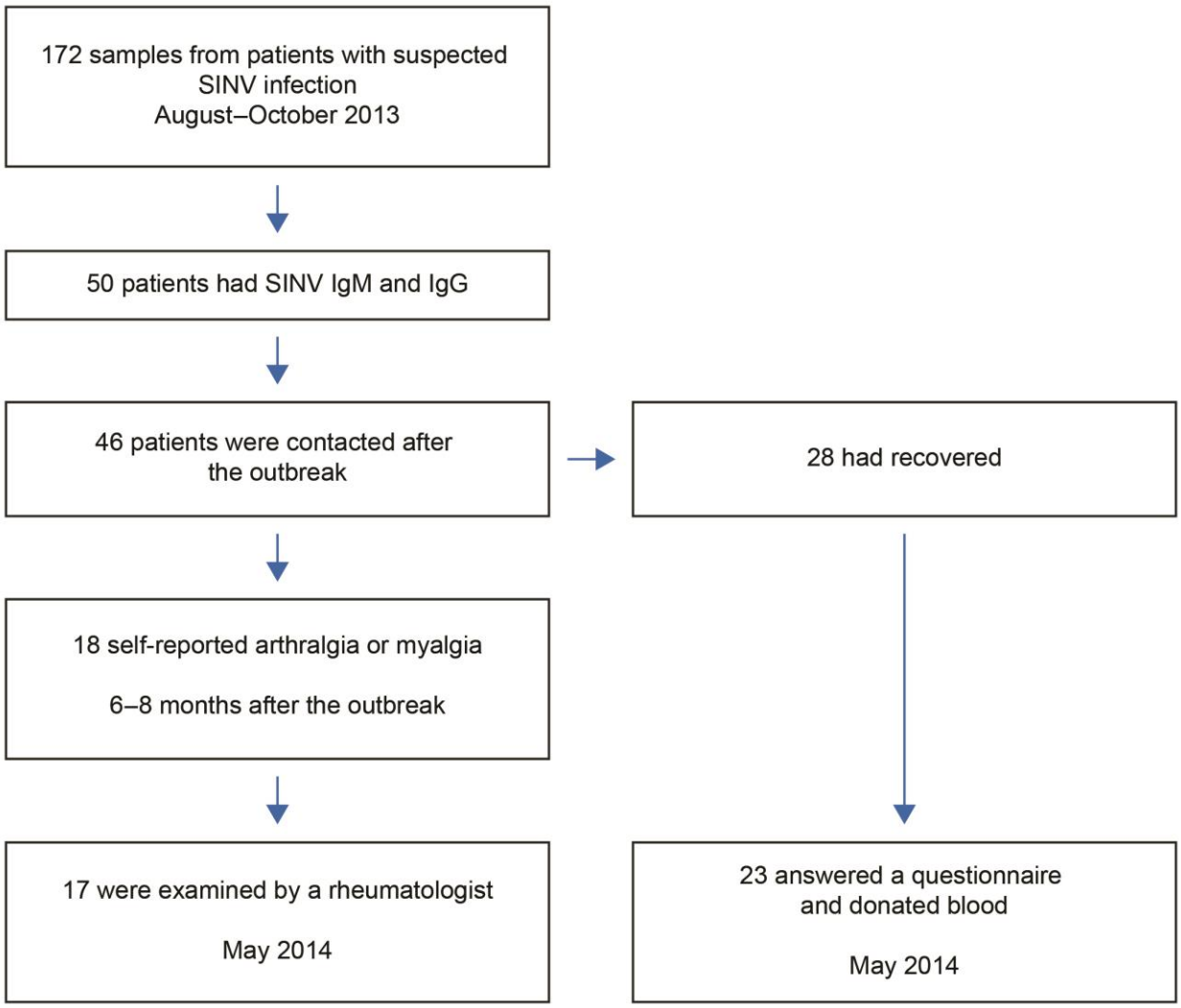
Characteristics	No. IgM and IgG positive	Median age, y (range)
Patient's sex (%)		
F	31 (62)	53 (33–85)
M	19 (38)	55 (28–72)
Geographic area		
Nonendemic area†	49	NA
Endemic area‡	1	NA

*50 patients had SINV-specific IgM and IgG in a single or a follow-up sample. NA, not applicable.
†The area in Sweden north of the 63rd parallel.
‡The area in Sweden between the 60th and 63rd parallels.

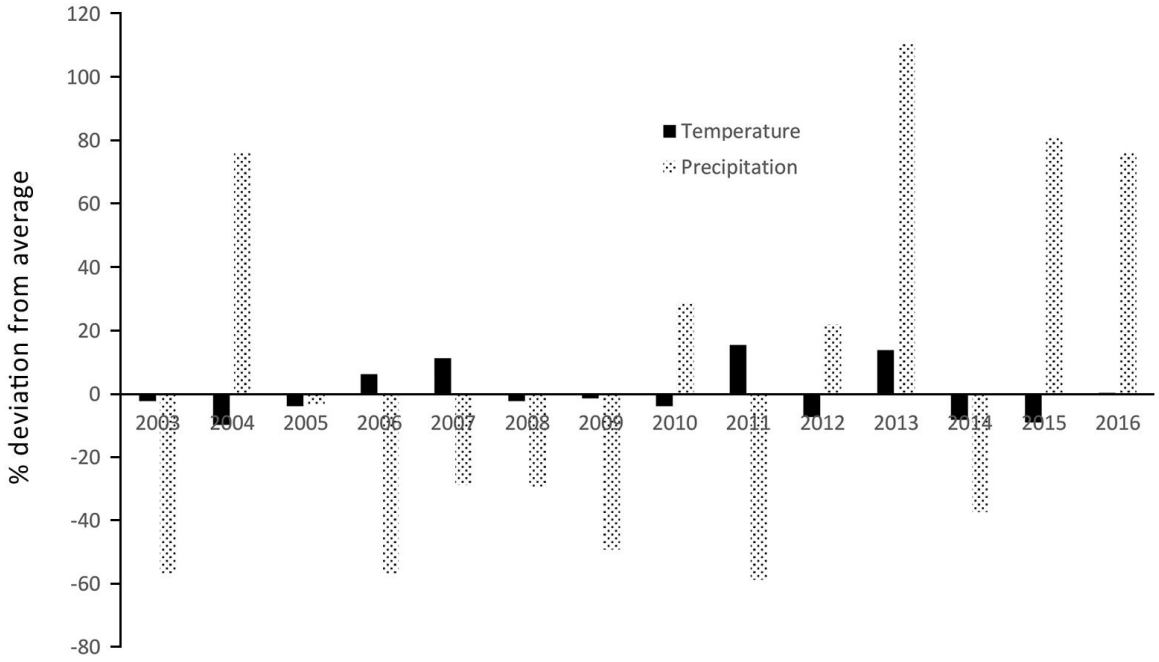
Technical Appendix Table 2. Follow-up information for patients with Sindbis virus 6–8 months after the outbreak, Sweden, 2014*

Characteristic	No. patients with persistent arthralgia/myalgia (n = 17)	No. patients who recovered (n = 23)
Patient characteristics		
Sex, no. (%)		
F	9 (53)	12 (52)
M	8 (47)	11 (48)
Median age, y (range)	54 (40–72)	ND
Clinical findings, no. (%)		
Arthritis in any joint of 66 joints assessed	1 (5.9)	ND
Any tender joint of 68 joints assessed	14 (82.4)	ND
Enthesitis/tendinitis/tenosynovitis	10 (58.8)	ND
Median no. tender joints/patient (range)	3 (0–38)	ND
Laboratory findings		
SINV IgM, no. (%)†	11 (65)	14 (61)
Rheuma factor positive, no. (%)‡	1 (5.9)	ND
Anti-citrullinated protein antibodies, no. (%)	0 (0)	ND
C-reactive protein (mg/L), median (range)	1 (0.6–3.4)	ND
Erythrocyte sedimentation rate (mm/h), median (range)	5 (1–21)	ND
Self-graded symptoms		
Pain VAS, mm§	36	10
Global disease activity VAS, mm§	31	6
Fatigue VAS, mm§	38	26
Health assessment questionnaire, 0–3 scale¶	0.38	0
Doctor´s global VAS, mm#	11	ND

*mm, millimeters; ND, not determined; VAS, visual analog scale
†IgM levels analyzed by enzyme immunoassay.
‡Rheuma factor >3.5 IU/mL is positive.
§Visual analog scale range 0–100 mm: pain VAS (patient assessment of pain), global disease activity VAS (patient's assessment of global health), fatigue VAS (patient's assessment of fatigue).
¶Health assessment questionnaire to investigate the patient's assessment of function (*8*).
#Doctor's assessment of the patient's global health.



Technical Appendix Figure 1. Flowchart of patient interactions in study of Sindbis virus, Sweden, 2013.



Technical Appendix Figure 2. High precipitation and high temperature in June at the weather station Bjuröklubb klubb (64°48′ N; 21°58′ E) in the middle of the outbreak area.