Epidemic Sindbis Virus Infection in Finland: A Population-Based Case-Control Study of Risk Factors

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Background. Sindbis virus (SINV) is an arthropod-borne alphavirus that causes rash and arthritis. In Finland, epidemics occur cyclically, but factors associated with clinical SINV infection are largely unknown. We conducted a population-based case-control study during the epidemic year 2002.

Methods. SINV cases were serologically confirmed and reported to the National Infectious Disease Registry. Five control subjects, matched for age, sex, and residence, were selected from the National Population Information System. Data were collected using a self-administered mail survey. Conditional logistic regression models were used to identify independent risk factors; missing data were addressed using Bayesian full-likelihood modeling.

Results. A total of 337 case patients (58% female; age range, 1–94 y) and 934 control subjects were enrolled. Reported exposure to mosquito bites (matched odds ratio [mOR], 16.7; 95% confidence interval [CI], 9.1–33.4) and spending time in woods or marshland (mOR, 1.8; 95% CI, 1.3–2.5) were independently associated with SINV infection in the multivariable model. The population-attributable risk for mosquito bites was 87.2%. There were dose-response relations for increased number of insect bites (mOR, 23.8–72.5) and increased time spent in woods or marshland (mOR, 1.3–2.2).

Conclusions. Educating the public in endemic areas to avoid mosquito exposure and use protective measures remain important prevention measures for SINV infection.
reported cases [3, 12]. The reasons for this peculiar epidemic pattern are unknown. Some studies have hypothesized that grouse may play a role in the occurrence of SINV outbreaks, because SINV outbreaks have previously coincided with decreases in grouse populations [13] and antibodies to SINV have been detected in grouse [14, 15]. Changes in climatic and weather conditions may also contribute to the epidemiologic patterns of SINV [14].

Factors associated with clinical SINV infection are largely unknown and have not been previously evaluated in controlled studies. To determine factors associated with acquiring acute SINV infection and to identify opportunities for prevention, we conducted a population-based case-control study during the epidemic year 2002. Information was also collected on clinical features of the disease and patient characteristics.

MATERIALS AND METHODS

Population-Based Laboratory Surveillance for SINV Infection

In Finland, serologically confirmed SINV infection is a reportable disease by the diagnosing laboratory. During the study period, 2 accredited reference laboratories in Finland (HUSLAB, Helsinki, and Department of Virology, University of Turku) performed serologic testing for SINV. Cases of SINV infection that were serologically confirmed and notified to the National Infectious Disease Registry (NIDR) were included in this study. Cases reported from one healthcare district (North Karelia) were excluded because of another ongoing study on SINV infection in the area [8].

Case-Control Study

Definition and Identification of Case Patients. A case patient was defined as a person with rash-arthritis illness in whom the diagnosis of SINV infection was confirmed by serology and reported to the NIDR between 15 July and 22 October 2002. The diagnostic criteria were seroconversion in paired serum samples and/or positive immunoglobulin (Ig) M result in enzyme immunoassay in a single serum sample [16]. At HUSLAB, the presence of SINV antibodies was also confirmed using hemagglutination inhibition test [16]. Case patients were excluded from the case-control study if they reported a previous physician-diagnosed SINV infection or had been absent from their permanent place of residence for >1 day during the 10-day exposure period before the onset of first symptoms of SINV.

Selection of Control Subjects. Healthy control subjects were identified among the general population. For each enrolled case patient, a list of persons who matched the patient according to year of birth, sex, and postal code of residency was generated from the National Population Information System. Control subjects were excluded from the study if they reported a rash illness or new joint symptoms (arthralgia) during the 2 months before data collection or a previous physician-diagnosed SINV infection. In addition, subjects were excluded if they were away from their permanent place of residence for >1 day during the 10-day period before the onset of illness in the respective case patients.

Data Collection

A self-administered, standard questionnaire was mailed to case patients and to 5 randomly selected control subjects who met the matching criteria. A reminder with the questionnaire was mailed to nonresponders twice. For children <15 years of age, the primary caretakers were asked to complete the questionnaire. Study participants were asked about demographic and household information, symptoms, and treatment of illness, physician visits, occupational (logging, gardening, farming) and leisure (hiking, camping, hunting) outdoor activities, animal contacts, untreated water exposure, insect bites, and measures used to protect against insect bites. For the case patients, the questions referred to the 10 days before onset of SINV infection (the exposure period), and for the control subjects, the 10-day period before the date when the first serum specimen was obtained from their respective case patients. The median duration between the onset of symptoms in the case patients and completing the questionnaire was 32 days (range, 13–123). Participants were asked to use a calendar as a memory aid to recall their activities and potential exposures.

Informed consent was obtained from the study subjects or their caretakers. Because this study was conducted as part of an investigation in response to an acute public health problem, the Finnish Ministry of Health determined that no ethics committee review was required.

Statistical Analyses

Matched odds ratios (mORs) and 95% confidence intervals (CIs) in univariate analyses were calculated using Cochran and Mantel-Haenszel statistics. To identify independent factors associated with SINV infection, we developed conditional logistic regression models. Because of the self-administered survey design, the variables had varying proportions of missing responses. In the univariate analyses, we assumed that missing data were missing approximately at random [17, 18]. Accordingly, only cases with complete information were included in these analyses.

We used P value of .15 as the screening criterion for selection of variables for the multivariable analyses. Statistical significance was considered at 5% level. The first multivariable analysis (model 1), was conducted using frequentist, non-Bayesian conditional logistic regression in which missing data were assumed to be missing completely at random [17, 18]. Only cases with complete information were included in model 1. To determine the best model, we used backward elimination. The main variable of interest was mosquito bites. The likelihood
ratio test was used to assess the statistical significance of each variable. All reported P values are 2-tailed.

We addressed the issue of missing data by using Bayesian full-likelihood modeling (conditional logistic regression, model 2) where the missing data becomes an additional parameter and the influence of missing data is taken into account in the model [19]. We also included variable selection indicators in the model and performed a Gibbs’ variable selection [19]. Only significant variables with >50% probability for inclusion were in the final model. The inclusion probabilities were derived from a Bayesian mean model that included the variables previously included in model 1.

We conducted a dose-response analysis for the variables that were significant in multivariable model 2: exposure to insect bites and spending time in woods or marshland. The dose was defined as the average number of insect bites (or hours of outdoor activity) per day multiplied by the number of days of exposure. The dose variables were treated as ordinal and quartiles of doses were used as cutoffs. The dose-response analysis was first done by frequentist conditional logistic regression (univariate); subsequently, significant variables were included in Bayesian conditional logistic regression model (multivariable).

Descriptive, univariate, and multivariable (model 1) analyses were completed using SPSS software (version 17; United States); Bayesian full-likelihood modeling was performed with Winbugs software (version 1.4.3; United Kingdom) [20]. Adjusted population-attributable risk (PAR) was calculated as described elsewhere [21]. The 95% CIs for PAR were calculated with a method based on Bonferroni inequality [22].

RESULTS

In 2002, a total of 597 laboratory confirmed cases of SINV infection were reported to the NIDR in Finland (incidence, 11.5/100 000/y) [12]; 575 cases (96%) were reported from 15 July to 22 October 2002. The incidence of infection peaked in September during week 36 (Figure 1). Case patients were identified mainly from 4 health districts with high rates of SINV infection in 2002 [12]. Residents of North Karelia health district, who were excluded from the study, accounted for 140 (23.5%) of reported cases.

Study questionnaires were returned by 369 of 391 eligible case patients (94.4%) and 1216 of 1832 control subjects (66.4%); 32 case patients and 134 control subjects were excluded according to the study protocol, mainly because of absence from the place of residence during the 10-day exposure period. Additional control subjects were removed owing to the exclusion of their respective case patients. A total of 337 case patients and 934 control subjects were included in the analyses. The median age for case patients was 49 years (range, 1–94 y); 58% were female.

Clinical Characteristics of Case Patients With SINV Infection

The most common symptoms of SINV infection were papular rash, joint symptoms, fatigue, muscle pain, and headache (Table 1). Upper respiratory symptoms and fever were reported by 36% of case patients. Of underlying medical conditions or previous injury affecting joint or connective tissue, joint injury

![Figure 1](image-url)  
**Figure 1.** Laboratory-confirmed cases of Sindbis virus infection reported to the National Infectious Disease Registry in Finland for 2002.
was reported significantly more often by case patients \((P = .02)\). Current or previous osteoarthritis and bacterial joint infection were also reported more commonly by case patients than by controls, but this difference was not statistically significant \((P = .06\) and .05, respectively) (Table 1).

Table 1. Clinical Symptoms and Previous or Current Medical Conditions of Case Patients With Laboratory-Confirmed Sindbis Virus Infection and Matched Control Subjects

<table>
<thead>
<tr>
<th>Symptoms and conditions</th>
<th>Case patients ((n = 337),) no. (%)</th>
<th>Control subjects ((n = 934),) no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rash or new joint symptoms</td>
<td>334/336 (99)</td>
<td>NA</td>
</tr>
<tr>
<td>Papular rash</td>
<td>321/334 (96)</td>
<td>...</td>
</tr>
<tr>
<td>Joint symptoms</td>
<td>322/335 (96)</td>
<td>...</td>
</tr>
<tr>
<td>Fatigue</td>
<td>238/308 (77)</td>
<td>36/873 (4.1)</td>
</tr>
<tr>
<td>Muscle pain</td>
<td>182/292 (62)</td>
<td>24/870 (2.8)</td>
</tr>
<tr>
<td>Headache</td>
<td>144/292 (49)</td>
<td>39/878 (4.4)</td>
</tr>
<tr>
<td>Neck pain</td>
<td>127/277 (46)</td>
<td>36/876 (4.1)</td>
</tr>
<tr>
<td>Fever (\text{temperature } &gt;38^\circ\text{C})</td>
<td>96/269 (36)</td>
<td>8/865 (1.1)</td>
</tr>
<tr>
<td>Lower back pain</td>
<td>93/266 (36)</td>
<td>25/870 (2.9)</td>
</tr>
<tr>
<td>Upper respiratory infection (\text{sore throat, rhinorrhea, cough})</td>
<td>109/301 (36)</td>
<td>49/893 (5.5)</td>
</tr>
<tr>
<td>Dizziness</td>
<td>62/267 (23)</td>
<td>20/866 (2.3)</td>
</tr>
<tr>
<td>Upper back pain</td>
<td>52/260 (20)</td>
<td>8/862 (.9)</td>
</tr>
<tr>
<td>Nausea</td>
<td>49/269 (18)</td>
<td>12/866 (1.4)</td>
</tr>
<tr>
<td>Enlarged lymph nodes</td>
<td>40/242 (16.6)</td>
<td>7/876 (.8)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>38/269 (14)</td>
<td>17/868 (2)</td>
</tr>
<tr>
<td>Photophobia</td>
<td>34/264 (13)</td>
<td>6/862 (.7)</td>
</tr>
<tr>
<td>Visual disturbances</td>
<td>20/257 (8)</td>
<td>6/861 (.7)</td>
</tr>
<tr>
<td>Previous or current medical conditions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Joint injury</td>
<td>69/311 (22)</td>
<td>124/843 (15)</td>
</tr>
<tr>
<td>Osteoarthritis</td>
<td>50/301 (17)</td>
<td>110/857 (13)</td>
</tr>
<tr>
<td>Bacterial joint infection</td>
<td>9/294 (3)</td>
<td>10/823 (1.2)</td>
</tr>
</tbody>
</table>

**NOTE:** NA, not applicable \((\text{exclusion criteria for control subjects})\).

Factors Associated With SINV Infection

**Univariate Analyses.** Case patients were significantly more likely to report bites by any insect compared with controls \((\text{mOR}, 31.8; \text{95\% CI, 11.5–87.8})\) (Table 2). However, of the specific arthropods, only mosquito bites were significantly associated with SINV infection; 93.5\% of case patients reported mosquito bites compared with 48.5\% of controls \((\text{mOR}, 19.7; \text{95\% CI, 9.0–43.1})\) (Table 2). Deer fly bites appeared to be associated with decreased likelihood of SINV infection (Table 2). Reported protective measures against insect bites, including insect repellents, mosquito coils, and nets, were not associated with a decreased likelihood for SINV infection (Table 2).

Most outdoor activities were significantly associated with SINV infection in univariate analyses (Table 3). Handling sick or dead animals \((\text{mOR}, 1.8; \text{95\% CI, 1.1–3.1})\) or having observed them near the residence \((\text{mOR}, 1.5; \text{95\% CI, 1.1–2.3})\) were also associated with SINV infection.

**Multivariable Analyses.** In model 1, exposure to mosquito bites was the only variable significantly associated with SINV infection \((\text{mOR}, 29.3; \text{95\% CI, 10.7–80.0})\) (Table 4). In model 2, spending time outdoors in the woods or marshland was also independently associated with increased odds of disease \((\text{mOR},...
1.8; 95% CI, 1.3–2.5), in addition to mosquito bites (mOR, 16.7; 95% CI, 9.1–33.4) (Table 4). The number of case patients and control subjects included in the analysis was substantially greater in the Bayesian model 2 where the missing data excluded from model 1 was taken into account (Table 4). No significant interaction was found between exposure to mosquito bites and spending time outdoors in woods or marshland. The adjusted PAR for mosquito bites in the multivariable model was 87.2% (.872; 95% CI, .78–.94).

**DISCUSSION**

This population-based study represents the first controlled evaluation of risk factors for SINV infection and also one of the largest reported studies of risk factors for arthropod-borne viral diseases. Few controlled studies have previously been conducted to assess risk factors for arthropod-borne viral diseases [23–25] and only one such study of alphaviruses has been reported [26]. Our data suggest that mosquito bites are the main mechanism of transmission for SINV infection with 87% of disease being attributable to mosquito bites. We also found a significant dose-response relation for increased number of all insect bites, reflecting primarily mosquito bites.

Previously, the late summer mosquito species, *Culex* and *Culiseta*, have been suspected to be potential vectors of SINV because the virus was isolated from these mosquitoes in Sweden.
in the 1980s [27, 28] and because most clinical cases occur during August and September [12, 14]. However, SINV was also isolated earlier from ticks in Italy [29] and it has been unclear whether other arthropods could transmit the disease, particularly because other viruses circulating between mosquitoes and birds, such as West Nile virus, can also be transmitted by ticks [30]. There was no suggestion in our data that bites from arthropods other than mosquitoes were associated with infection. Because no antiviral or vaccine is available for treatment and prevention of SINV infection, our results indicate that educating the public about protecting against mosquito bites during outdoor activities in endemic and mosquito-abundant areas is important to avoid the infection.

In addition to mosquito bites, spending time outdoors in woods or marshland was independently associated with SINV infection in model 2 with a significant dose-response relation. About two-thirds of case patients reported spending time in woods or marshland, which is probably a proxy for mosquito exposure. Some participants may not have noticed being bitten by mosquitoes while spending time outdoors.

The following limitations should be considered when interpreting the results. Although the control subjects who reported rash-arthritis illness were excluded from the study, we did not test control subjects for SINV antibodies and some individuals with SINV infection may have been included among control subjects, resulting in misclassification, particularly because mild and asymptomatic infections are common [14]. However, this misclassification would probably be nondifferential and would have made detecting an association more difficult. Due to the highly specific (95.2% and 97.6% for IgM and IgG, respectively) and sensitive (97.6% and 100% for IgM and IgG, respectively) [16] enzyme immunoassay test used in laboratory confirmation, misclassification of disease status among case patients is unlikely. Although the case-control study design included features aimed at decreasing potential recall bias, such as using a calendar for memory aid, case patients may have had better

<table>
<thead>
<tr>
<th>Factor</th>
<th>Model 1</th>
<th>Model 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposure to mosquito bites</td>
<td>29.3 (10.7–80.0)</td>
<td>16.7 (9.1–33.4)</td>
</tr>
<tr>
<td>Spending time in woods or marshland</td>
<td>NS</td>
<td>1.8 (1.3–2.5)</td>
</tr>
<tr>
<td>Case patients/control subjects included in analysis, no.</td>
<td>194/339</td>
<td>322/934</td>
</tr>
</tbody>
</table>

**NOTE.** Model 1 (frequentist conditional logistic regression model) included the following variables: sleeping in a tent; spending time in woods or marshland; forestry work; gardening; land or field preparation; cleaning hay or grain; picking berries in forest; drinking unpurified water; swimming in a lake, river, or dam; handling sick or dead animals; having observed sick or dead animals in a close vicinity; wearing long pants; and exposure to mosquito bites. Model 2 (Bayesian full-likelihood conditional regression model) included the following variables: exposure to mosquito bites and spending time in woods or marshland; these variables were selected from the Bayesian mean model using Gibb’s selection. aOR, adjusted odds ratio; CI, confidence interval (model 1) or Bayesian credible interval (model 2); NS, not statistically significant.

**Figure 2.** Dose-response relations for number of insect bites and time spent outdoors versus odds of Sindbis virus infection. Data represent matched odds ratios of dose quartiles for insect bites (solid line with squares) and for time spent outdoors in woods or marshland (dashed line with circles), and error bars represent the 95% Bayesian credible intervals.
recall for exposure to mosquitoes than control subjects. However, strength of the association and presence of a significant dose-response relation with insect bites increases confidence in the finding. Additionally, differential recall among case patients and control subjects should probably not influence the other significant variable, time spent outdoors, to the same extent as mosquito bites.

Although exposure to other arthropod species was not associated with SINV infection, the study subjects may have had difficulty in identifying various insects, particularly those other than mosquitoes. The inverse association with deer fly bites in the univariate analysis may be a chance finding and related to the high degree of missing responses for this variable. Alternatively, this finding may be related to the fact that deer flies are a major nuisance in late summer and early autumn and people may therefore be more likely to wear protective clothing and nets in areas where deer flies are present, thus avoiding mosquito bites and, consequently, SINV infection.

Reported protective measures against insect bites were not significantly associated with reduced likelihood of SINV infection. In contrast, a case-control study of RRV in tropical Australia [26] found that protective measures such as mosquito coils, repellents, and light-colored clothing reduced disease. Additionally, in tropical countries, the use of insect repellents and nets to protect against mosquito-borne viruses, such as CHIKV and Dengue, is promoted by public health authorities [31, 32]. The reasons for lack of association in our study are unclear, but they may be related to difficulties in measuring the extent of protection, lack of statistical power due to relatively small number of study subjects reporting protective measures, or the fact that the use of protective measures against insect bites may be less common in Finland than in tropical regions, because general awareness of mosquito-borne pathogens in the population may be lower than in countries where life threatening, mosquito-borne illnesses are present.

The findings of our study add to the knowledge base of clinical features of SINV infection and are consistent with those of other studies regarding the main symptoms [6, 8, 9, 33, 34]. However, in contrast with previous reports, one-third of case patients reported upper respiratory infection symptoms. It is unclear whether these symptoms were from SINV infection or resulted from concurrent infection with respiratory viruses, as has been reported for CHIKV infection [35]. At the time when they completed the questionnaire, 69% of case patients reported ongoing symptoms, mostly joint symptoms; only 31% were able to report when joint symptoms ended. In contrast, almost 90% of the case patients were able to report the date when the rash subsided. These data further highlight that persistent joint symptoms are common among persons infected with SINV, contributing to the total disease burden and healthcare costs due to SINV and its sequelae.

Case patients had past or active joint injury significantly more often than controls; ongoing or past bacterial joint infection or osteoarthritis was also reported more often, although this difference was not statistically significant. Distinguishing joint symptoms due to these conditions from those of SINV infection may be difficult. The presence of underlying damage to the joint tissue may predispose to more severe SINV symptoms. Other possibilities include common genetic factors associated with both an increased susceptibility for osteoarthritis, which has a definite genetic compound [36], and symptomatic SINV infection. For other alphaviral diseases, genetic risk factors have been suggested including HLA DR7 haplotype for RRV [37] and Rh-positive blood group for CHIKV infection [38, 39].

An open-ended question about the probable day of exposure among case patients enabled us to estimate the median incubation period of SINV to be 4 days (range, 2–18 d), similar to those of other related alphaviruses, such as CHIKV and RRV [40, 41]. However, self-reported time of exposure may be inaccurate and should be interpreted with caution. Nevertheless, the previous estimate for the incubation period of SINV (8–9 d) was based on extremely limited data, basically from a single case report [8].

The median duration between the onset of SINV symptoms and the first medical contact was 2 days. Another study showed that IgM seroconversion occurs within the first 8 days after onset of symptoms and that only ~40% of the patients had positive IgM result in the first diagnostic serum sample [8]. Given that patients seem to seek medical care within a few days after onset of illness, a negative SINV antibody result during the first week of disease is probable, requiring a convalescent serum sample to confirm the diagnosis.

We addressed the problem of missing information in a self-reported survey by using a Bayesian full-likelihood modeling approach [19] and noted that for some covariates data were not missing completely at random, as assumed in frequentist conditional logistic regression. Bayesian models are less sensitive to bias caused by missing data [19], and the results were slightly different from those of the frequentist model, because of accounting for the missing data and increased statistical power. The 2 variables found to be significant in the Bayesian model were also independently significant in a frequentist model which included only these variables (data not shown), suggesting that having additional variables in model 1, where missing data were not addressed, may have resulted in a biased estimates. These results highlight the advantage of using current statistical methods for dealing with missing information in self-reported surveys to increase the validity of results.

In conclusion, this population-based study adds information about risk factors for SINV infection and provides a comprehensive description of its clinical characteristics. Given the high incidence of SINV in Finland [15], future studies should address the full disease burden and the public health and economic...
impact of SINV epidemics, particularly the long-term effects of persistent joint symptoms on the quality of life.

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References