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Chikungunya Virus in North-Eastern Italy: A Seroprevalence Survey

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Abstract. After an outbreak of Chikungunya infection in Emilia-Romagna Region (North-eastern Italy), a survey was performed to estimate the seroprevalence of antibody to Chikungunya virus and the proportion of asymptomatic infections, to identify factors associated with infection, and evaluate the performance of the surveillance system. The method used was a survey on a random sample of residents of the village with the largest number of reported cases. The prevalence was 10.2% (33 of 325), being higher in older people and males, and lower when window screens and insect repellents were used. Only 18% of infected persons were fully asymptomatic, 85% of the 27 symptomatic confirmed cases satisfied the surveillance case definition, and 63% of the persons meeting the criteria for suspect case were identified by the active surveillance system. This study provides basic parameters for modeling the transmission potential of outbreaks and planning control measures for Chikungunya infection in temperate settings.

INTRODUCTION

Chikungunya is an arboviral disease caused by a virus of the Alphavirus genus in the *Togaviridae* family, which is transmitted by *Aedes* spp. mosquitoes. Chikungunya virus is usually endemic in several areas of rural Africa where there are numerous animal reservoirs and a continuous transmission to human beings. Conversely, large epidemics have been observed in Asian urban areas, involving populations with weak herd immunity. During the epidemic periods, human beings are the main virus reservoir.[1]

A Chikungunya outbreak was recently reported in Emilia-Romagna Region (North-eastern Italy) in the summer of 2007.[2] This was the first epidemic reported in a temperate area; the affected area has been known for many years as a region with a high concentration of *Aedes albopictus*.[3] The index case was a man coming from an epidemic area (Kerala, India) and the local transmission was sustained by *Ae. albopictus*. The outbreak mainly affected two small villages divided by a river (Castiglione di Cervia and Castiglione di Ravenna), but small clusters were also detected in other parts of the Region. An active surveillance system for the identification of all suspected cases (patients with fever and joint pain), based on weekly telephone calls to general practitioners (GPs) and emergency departments, was implemented during the event. Suspected cases were confirmed using molecular and/or serological tests. We report the results of a seroprevalence survey aimed at estimating the frequency of infected persons and the proportion of asymptomatic infections of the epidemic in Castiglione di Cervia, to identify factors associated with Chikungunya infection, and to evaluate the performance of the surveillance system.

MATERIALS AND METHODS

The two neighboring villages, where the outbreak occurred, are situated 6 km from the Adriatic coast; houses are typically low, surrounded by small gardens full of flowers, plants, and flowerpots. The study was run between 3 and 5 months after the end of the epidemic. A representative sample of residents of Castiglione di Cervia village (total population about 2,000 inhabitants) was randomly selected from the census list and stratified by age category (0–19, 20–39, 40–59, 60–79, and ≥ 80 years of age). A study sample size of 325 subjects was calculated to obtain 3% absolute precision, with a 95% confidence level, based on an estimated attack rate of 10%. Non-respondent individuals were replaced by others selected randomly. A standardized telephone questionnaire delivered by a restricted number of trained interviewers was used. The questionnaire was constructed to gather information about individual and household characteristics, symptoms during the epidemic period compatible with Chikungunya infection, exposure to mosquitoes, and knowledge, attitudes, and practices concerning Chikungunya infection and prevention of mosquito-transmitted diseases. As symptoms (especially fever) were collected retrospectively, they were defined in general terms only (i.e., presence/absence). All selected individuals were requested to submit to testing for Chikungunya serology. Tests were performed by the indirect immunofluorescence method[4]; subjects with a IgG titer ≥ 1:100 were classified as seropositive. Using a total of 65 serum samples obtained from either patients or vaccinated volunteers, the indirect fluorescent antibody (IFA) test used in this study was checked for potential cross-reactivity with the following viruses: Yellow fever (n = 8), Rubella (n = 15), Dengue (n = 10), Hepatitis C (n = 25), West Nile virus (n = 7). None of these samples gave a positive reaction (titer ≥ 1/100 for IgG and titer ≥ 1/50 for IgM antibody response). Thus, the antibody titers detected can be assumed to be likely caused by a specific response to past Chikungunya virus (CHIKV) infection. The study was subjected to the Romagna

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Ethic Committee for approval, and all participants signed an informed consent form.

Data were analyzed using the SAS software system (version 9.0, SAS Institute, Cary, NC). A significance level of 0.05 was used throughout. Association of categorical variables with positive Chikungunya serology was assessed by the χ^2 test or Fisher's exact test, as needed. Multivariate analysis was performed by unconditional logistic regression using the Wald test; variables associated with P value ≤ 0.2 in the univariate analysis were included in the multivariate analysis. Model fitting was evaluated by the likelihood ratio χ^2 test and analysis of discordant pairs.

RESULTS

The final study population included 325 individuals. One hundred nineteen subjects were replaced during the survey either because they refused to be interviewed ($N = 38$) or they could not be contacted ($N = 81$). The target and the final sampled populations did not differ by age or sex. The prevalence of antibodies against Chikungunya virus in Castiglione di Cervia was 10.2% (33 cases out of 325). The prevalence of antibody to CHIK virus significantly increased with age and was higher in men than women (Table 1). In 23 people with antibodies to Chikungunya virus (70%), clinical presentation matched the definition of suspected cases (fever and joint pain) used for active surveillance during the outbreak, whereas 4 (12%) had fever or joint pain and 6 (18%) were fully asymptomatic (Table 2). Therefore, 23 of 27 symptomatic people matched the case definition used during the outbreak (85%). All asymptomatic individuals with positive antibody titers were male and two were born and had recently traveled (≤ 2 years before the outbreak) in Senegal. In the univariate analysis, the prevalence of infection was higher in older age groups, and in males, whereas it was lower among individuals using window screens and insect repellents during the epidemic. Continuous presence in Castiglione during the outbreak period, use of pest control measures, and work or recreational activities outside Castiglione were unevenly distributed among infected and noninfected individuals, but these differences were not statistically significant. Older people were significantly less likely to use insect repellents and to have been out of Castiglione di Cervia for work or recreational reasons; the odds ratios for one increase in age were 0.98 ($P < 0.001$) for both variables. None of the symptomatic individuals had traveled to Chikungunya endemic areas. The multivariate analysis showed that the prevalence of infection increased with age (2% increase for each additional year) and among males (borderline significant level increase), whereas it decreased in people claiming to have window screens (53% significant decrease), and to use insect repellents (borderline significant level decrease) (Table 3).

Twelve of 33 persons with antibodies to Chikungunya virus were not reported during the epidemic: five matched the definition of suspected cases used for active surveillance, two presented only fever, and five were fully asymptomatic. Of the 325 interviewed individuals, 30 reported having symptoms at the time the outbreak occurred: 23 were confirmed seropositive (18 identified and confirmed during the epidemic, one reported during the epidemic but seronegative at the initial test, four first identified during the serosurvey), whereas seven, despite having symptoms, were not identified by active surveillance and were later found to be seronegative. Therefore, of all symptomatic individuals matching the criteria for suspected cases during the outbreak, only 19 (63%) were correctly identified by active surveillance and tested for Chikungunya infections during the outbreak.

TABLE 1
Prevalence of antibodies to Chikungunya virus in Castiglione di Cervia*

	Survey population (n =)	Prevalence rates		
		No. of infected persons	%	95% CI
Total population	325	33	10.2	7.1–14
Age category (years)				
0–19	44	1	2.3	0.1–12
20–39	71	3	4.2	0.9–11.9
40–59	99	13	13.1	7.2–21.4
60–79	88	11	12.5	6.4–21.3
≥ 80	23	5	21.7	7.5–43.7
Gender				
Female	170	12	7.1	3.7–12
Male	155	21	13.5	8.6–20

* CI = confidence interval.

TABLE 2
Clinical presentation of Chikungunya infections

	No. infected persons	%
Clinical presentation		
Fever + joint pain (±other symptoms)	23	69.7
Fever	2	6.1
Joint pain + rash + other symptoms (except fever)	2	6.1
No symptoms	6	18.2
Single symptoms		
Fever	25	75.8
Joint pain	25	75.8
Fatigue	21	63.6
Muscle pain	15	45.5
Headache	15	45.5
Cutaneous rash	10	30.3
Others	20	60.6

DISCUSSION

The seroprevalence of Chikungunya infection in Castiglione di Cervia detected after the epidemic was lower than rates observed in other similar events: in the Reunion Island epidemic of 2006, Gerradin and others[5] reported a population seroprevalence equal to 38.2%, and Sossoko and others[6] equal to 37.2%. This may be caused by several factors, including differences in the biology and ecology of *Ae. albopictus* in Italy compared with Reunion Island. In addition, a possible explanation of this finding is the timeliness and effectiveness of measures to control vectors implemented during the event: fast-acting insecticides (synergized pyrethrins) were used for 3 days consecutively, sprayed with a truck-mounted atomizer in public areas (the only method allowed in Italy to apply pyrethrins in public areas), and a backpack mist blower in private spaces. Antilarval measures, using formulations of insect growth regulators and *Bacillus thuringiensis* var. *israeliensis* were also implemented. House-to-house interventions were implemented to eliminate breeding places, and community participation was encouraged. These control measures were carried out within a radius of 100 m of the residence of

TABLE 3
Univariate and multivariate analysis of association between characteristics and behavior of subjects and Chikungunya infection*

	Univariate analysis			Multivariate analysis†		
	OR	95% CI	*P* value	OR	95% CI	*P* value
Age	1.02‡	1.01–1.04	0.008	1.02‡	1.00–1.04	0.021
Male gender	2.06	0.98–4.35	0.057	1.94	0.90–4.19	0.093
Use of insect repellents	0.35	0.15–0.77	0.009	0.491	0.21–1.13	0.094
Use of window screens	0.43	0.21–0.89	0.023	0.47	0.22–0.99	0.047
Use of pest control measures	0.58	0.28–1.20	0.14			
Constant presence in Castiglione during the epidemic period	2.12	0.72–6.24	0.17			
Work or recreational activities conducted outside Castiglione	0.60	0.29–1.27	0.18			

*The table only includes variables which, in the univariate analysis, are associated with the outcome with *P* value ≤ 0.2. OR = odds ratio; CI = confidence interval.
†All the variables listed in the table were included in the multivariate analysis.
‡Odds ratio for a 1 year increase in age.

each suspected infection case; for clusters, the control measures were extended to a 300-m radius of the most external case. The hypothesis of the positive effect of the aforementioned control measures is supported by a recent study, based on mathematical modeling, which showed that the attack rate in the Castiglione di Cervia epidemic would have been much higher in the absence of effective control measures (including information campaign).[7]

However, other factors such as the influence of climatic conditions cannot be ruled out. On Reunion Island, despite comprehensive efforts, the substantial decline in the number of incident cases was observed only at the onset of southern hemisphere winter, when climatic conditions were less favorable to the multiplication and activity of the vector.[8] Accordingly, the reproduction rate in Reunion Island has been reported to vary according to the season.[9] Thus, the influence of climatic conditions, such as the effect of colder winters in temperate regions, should also be considered as critical in the dynamics of such outbreaks.

The low proportion of asymptomatic infections (18%) and the observed ratio of symptomatic to asymptomatic infections equal to 4.5 is close to that found in a previous epidemic,[5] confirming that, in areas where surveillance systems are effective, epidemic detection can be timely and control measures appropriately implemented. Moreover, the proportion of asymptomatic infections (and also the prevalence rate) could have been oversized because of the presence of two subjects who were born and had traveled during the previous 2 years in an endemic area (Senegal). These subjects might have been previously infected in Senegal without presenting symptoms during the Castiglione epidemic, despite likely renewed contact with Chikungunya virus that could have boosted specific immunity in at least one subject with a high IgG titer.

The proportion of infected persons with typical clinical presentation (fever and joint pain ± other symptoms) and the ratio of infected males to infected females are different from that reported in a previous work on the same epidemic[2] because asymptomatic infections were also detected in the present survey. In this work, the proportion of persons that had symptoms during the outbreak and were seropositive for antibodies to Chikungunya virus matching the definition of suspect case (fever and joint pain) was 70% (85% if only symptomatic cases were considered). Considering all (confirmed or not) cases, the probability of these cases being detected by active surveillance was 63% (19 out of 30). This performance, which is intermediate between the Reunion Island epidemic, where more intensive active surveillance was implemented, and the Mayotte Islands epidemic,[10] is probably acceptable given the limited resources and practical limitations of public health surveillance.

This serosurvey shows that age and gender were associated with the likelihood of infection: both older people and males had a higher probability of being infected, although the latter association was only borderline significant in the multivariate analysis. Because there is no plausible biological explanation for this finding, the more likely reason is that age and gender are proxy-factors for specific behavior that causes higher exposure to *Ae. albopictus* bites (i.e., staying outdoors in Castiglione di Cervia during daytime) and less tendency toward individual protection (i.e., use of insect repellents). Although the use of insect repellents was controlled in the multivariate analysis, it is possible that there remains residual confounding of the relationship between age and infection and between sex and infection. For example, analytical information on the characteristics of insect repellents (i.e., typology, period of use, number of applications per day) and about use of insect repellent was not collected and therefore not accounted for in the analysis. The same applies to other risk-specific behaviors that were only partially captured by the study questionnaire, which was short because it was administered by a telephone interview. For instance, information was not analytically collected on the extent to which work and recreational activities, during the epidemic and outside Castiglione, occurred in the daytime when there was higher risk of *Aedes mosquito* bites and whether people, who reported having always stayed in Castiglione, actually spent time outdoors in the day during prevalent time of *Ae. albopictus* activity.

Another interesting finding is the protective effect of window screens, which were not expected to reduce the risk of infection caused by the preference of *Ae. albopictus* to bite outdoors. However, the use of screens may be a possible proxy measure of individual care in preventing mosquito bites or perhaps an indication of modified behavior in *Ae. albopictus*, with an increased tendency to bite indoors, although this fact can only be proven by appropriate entomological studies. Finally, no association was found between the characteristics of buildings and risk of infection; this is probably intrinsic in the structure of the village where the epidemic occurred: all the houses in Castiglione di Cervia are very uniform (buildings with one or two floors surrounded by a small garden).

In conclusion, this serosurvey provides valuable insights on Chikungunya transmission in a nonendemic country like Italy: symptomatic infections represent the majority of all infected persons; a case definition based on fever plus joint pain and/or other symptoms can correctly identify 85% of symptomatic cases; active surveillance based on weekly telephone calls

to GPs is reasonably effective in identifying more than two-thirds of new cases. Moreover, results of this study suggest that the low attack rates, less than 40 years of age reported during the outbreak, were not attributable to different host response, but simply to behavioral factors leading to differential exposure to mosquitoes.[2]

These parameters are of paramount importance for modeling dynamics of Chikungunya and planning adequate control measures in temperate areas, because no previous data on attack rates, correlates of infection, and proportion of asymptomatic infections were available in these settings.

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