patients are currently in an isolation ward.

Virologists say previous strains destroyed the linings of blood capillaries and vessels, prompting fluids to drain out of the circulatory system through the body's orifices and pores, killing the victim through shock. But there is not much bleeding in the new strain that appears to kill its victims after provoking a high fever, they say.

Authorities have assembled epidemiologists and virologists in the affected district to monitor the disease. "We have put our people on alert for anyone who is complaining of fever, abdominal pain, vomiting and has developed rashes," Okware said, referring to the early symptoms of the disease caused by the new strain.

An outbreak of Ebola, a highly contagious disease that can have fatality rates as high as 90 percent, killed at least 170 people in northern Uganda's Gulu district in 2000. A similar outbreak has killed at least 26 people in the West Kasai region of the Democratic Republic of Congo in recent weeks, according to the country's Health Minister Victor Makwenge Kaput.

It spreads by direct human contact, especially through infected blood. The Ebola virus was first identified in 1976 in Sudan and in a nearby region of Democratic Republic of the Congo (then Zaire). Outbreaks of Ebola have also occurred in the Ivory Coast and Gabon.

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[The difference in symptoms of the disease associated with this new strain of Ebola virus, and its less hemorrhagic presentation, may account in part for the delay in identifying the causes of the outbreak. On the basis of the WHO report it must be presumed that the disease was present in the region for several weeks prior to recognition of the 1st case on 10 Nov 2007.

An interactive map of the Bundibugyo region of Western Uganda is available at <http://www.maplandia.com/uganda/bundibugyo/>. - Mod.CP]

[see also:
Hemorrhagic fever - Uganda (04): (Bundibugyo), Ebola confirmed 20071130.3859
Hemorrhagic fever - Uganda (03): (Bundibugyo) 20071121.3775
Hemorrhagic fever - Uganda (02): (Bundibugyo), Marburg NOT 20071116.3718
Hemorrhagic fever - Uganda (Bundibugyo): Marburg susp., RFI 20071114.3697
Marburg hemorrhagic fever - Uganda (06): new case 20071002.3257
Marburg hemorrhagic fever - Uganda (05) 20070817.2697
Marburg hemorrhagic fever - Uganda (04), WHO 20070814.2656
Marburg hemorrhagic fever - Uganda (03) 20070810.2609
Marburg hemorrhagic fever - Uganda (02) 20070804.2533
Marburg hemorrhagic fever - Uganda 20070803.2514
Viral hemorrhagic fever - Uganda (Kamwenge): Marburg conf. 20070801.2490
Viral hemorrhagic fever - Uganda (Kamwenge): Marburg susp., RFI 20070801.247
2001

Ebola hemorrhagic fever - Uganda: postscript 20011019.2576]
.....cp/ejp/lm

別紙様式第2-1

No. 14

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研究報告の概要

○イタリアでのチクングニヤウイルス感染症:温帯地域におけるアウトブレイク

背景:ヤブカ類(Aedes spp.)によって伝播されるチクングニヤウイルス(CHIKV)は、近年インド洋諸島やインド亜大陸において複数のアウトブレイクを引き起こした。ここではイタリアでのアウトブレイクを報告する。

方法:イタリア北東部の隣りあった2つの村で原因不明の発熱性疾患が多数報告された後、主要感染源と伝播形態を特定するためのアウトブレイク調査を実施した。能動的サーベイランスシステムも導入した。臨床症例定義は、発熱と関節痛の発症とした。血液検体を採取、PCRと血清学的検査を行って病原体を特定した。現地で採取した蚊にもPCRを実施した。CHIKV E1領域の系統発生学的解析を行った。

知見:ヒトおよび蚊由来検体の分析により、当該アウトブレイクはCHIKVによるものと判明した。2007年7月4日から9月27日までにCHIKV感染症例205例が特定された。推定初発症例は、当該の村に親類を訪ねた際に発症したインドの男性とされた。系統発生学的解析では、イタリアで特定された株とこれより前にインド洋諸島のアウトブレイク時に特定された株との間に高い相同性が示された。ほぼ全例とも症状はかなり軽度で、死亡報告は1例のみであった。

考察:非熱帯地域における今回のCHIKV感染症アウトブレイクは、ある意味予期せぬ事態であり、グローバル化時代における新興感染症の脅威に対する準備と対策の必要性が強く示唆される。

報告企業の意見	今後の対応
2007年7月4日から9月27日までにイタリアにおいてチクングニヤウイルス感染症例205例が集団発生し、グローバル化時代における新興感染症の脅威に対する準備と対策の必要があるとの報告である。	日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。国内でチクングニヤ熱が確認されたため、渡航歴確認の徹底を図っている。また、チクングニヤ熱の既往歴がある場合、治癒後6ヵ月間は献血不適としている。今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。

使用上の注意記載状況・その他参考事項等

合成血「日赤」
照射合成血「日赤」
合成血-LR「日赤」
照射合成血-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

Infection with chikungunya virus in Italy: an outbreak in a temperate region

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See Comment page 1805

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Summary

Background Chikungunya virus (CHIKV), which is transmitted by *Aedes* spp mosquitoes, has recently caused several outbreaks on islands in the Indian Ocean and on the Indian subcontinent. We report on an outbreak in Italy.

Methods After reports of a large number of cases of febrile illness of unknown origin in two contiguous villages in northeastern Italy, an outbreak investigation was done to identify the primary source of infection and modes of transmission. An active surveillance system was also implemented. The clinical case definition was presentation with fever and joint pain. Blood samples were gathered and analysed by PCR and serological assays to identify the causal agent. Locally captured mosquitoes were also tested by PCR. Phylogenetic analysis of the CHIKV E1 region was done.

Findings Analysis of samples from human beings and from mosquitoes showed that the outbreak was caused by CHIKV. We identified 205 cases of infection with CHIKV between July 4 and Sept 27, 2007. The presumed index case was a man from India who developed symptoms while visiting relatives in one of the villages. Phylogenetic analysis showed a high similarity between the strains found in Italy and those identified during an earlier outbreak on islands in the Indian Ocean. The disease was fairly mild in nearly all cases, with only one reported death.

Interpretation This outbreak of CHIKV disease in a non-tropical area was to some extent unexpected and emphasises the need for preparedness and response to emerging infectious threats in the era of globalisation.

Introduction

Chikungunya virus (CHIKV) is an arthropod-borne virus transmitted to human beings by *Aedes* spp mosquitoes. After the isolation of the virus in Tanzania in 1953,[1] sporadic cases and a number of outbreaks of infection with CHIKV have been reported in several African countries, on the Indian subcontinent, and in southeast Asia.[2] In the past few years, a series of outbreaks have been reported over a large geographical area that includes African islands in the Indian Ocean and the Indian subcontinent. The first of the outbreaks occurred in Kenya in 2004, followed by outbreaks on the Comoros Islands, the island of La Réunion, and other islands in the southwest Indian Ocean in early 2005, and by a large outbreak in India in 2005–06.[3,4] According to the molecular analysis of the strains isolated on islands in the Indian Ocean and in India, the epidemic was caused by a variant of the central/east African genotype of CHIKV.[5]

During the outbreak on islands in the Indian Ocean, a large number of travellers from industrialised countries with a temperate climate became infected with CHIKV and were still infected on returning home.[6–9] In some of these industrialised countries, *Aedes albopictus*—a vector of CHIKV—was introduced a number of years ago and is now widespread,[10] with an especially high population density in Italy.[11] This situation is particularly threatening because it has been suggested that the strain of CHIKV in the Indian Ocean has better adapted to *A albopictus* than it has to other *Aedes* spp.[4] Nonetheless, to date, no outbreaks due to the local transmission of CHIKV have been reported in these countries. Here, we report on a large outbreak of CHIKV infection that occurred in two neighbouring villages in Italy.[12]

Methods

Patients

In July and August, 2007, the local health unit of the province of Ravenna (region of Emilia Romagna, northeastern Italy) detected an unusually high number of cases of febrile illness in Castiglione di Cervia and Castiglione di Ravenna, two small villages divided by a river. In the second week of August, the local health unit implemented an active surveillance system to identify, both prospectively and retrospectively, all individuals with febrile illness, on the basis of reports provided by general practitioners and hospital emergency units. Patient data were collected with a standardised questionnaire and included age, sex, place of residence, countries visited, travel dates, and date of onset of symptoms. In late August, an outbreak investigation was done to identify the agent and the source of the infection and to better understand the dynamics of the epidemic of febrile illness.

Early in the outbreak investigation, infection with CHIKV was suspected because of clinical symptoms and the fact that the first patient with febrile illness was a man from a country affected by an outbreak. Furthermore, the presence of *A albopictus* in the area was known. A case of CHIKV infection was defined as the presence of

high fever (>38·5°C) and joint pain and living in, or having visited, one of the two villages; for this definition, laboratory confirmation was not required. Individuals with fever but no joint pain and those with these symptoms who did not live in the villages or who had not visited them were deemed to be cases only if laboratory confirmation was obtained (ie, positivity to either haemagglutination inhibition or PCR).

Procedures

Blood and serum samples were stored at –80°C before being tested. Samples were tested for antibodies to CHIKV by haemagglutination inhibition and initially confirmed by plaque reduction neutralisation assays in 2 cases who were haemagglutination inhibition-positive; both tests were done at the Istituto Superiore di Sanità, Rome.[11] Thereafter, only the haemagglutination inhibition test was used. Samples were also tested for antibodies to dengue virus and yellow fever, also with the haemagglutination inhibition test.

To detect the presence of viral genomic RNA in human samples, real-time RT-PCR targeting the *nsp1* gene of CHIKV was done. The assay was based on the Qiagen One Step RT-PCR kit, and a 25 µL reaction volume included 3 µL RNA extract (Qiagen Viral RNA Mini kit), 40 ng/µL bovine serum albumin, 400 µmol/L of each dNTP, 600 nmol/L CHIKV sense (tgatcccgactcaaccatcct), 600 nmol/L CHIKV anti-sense (ggcaaacgcagtggtacttcct) primers, and 200 nmol/L probe ChikP (FAM-tccgac-atcatcctccttgctggc-Black Hole Quencher 1). Amplification was done in a Roche Light Cycler (Indianopolis, IN, USA) and involved the following steps: 50°C for 30 min, 95°C for 15 min, and 45 repetitions of 95°C for 15 s then 58°C for 30 s.

PCR was also used to detect CHIKV in specimens of *A albopictus* that were captured locally during the outbreak. Total RNA was extracted from the supernatant of an homogenate of mosquitoes in minimal essential medium, using TRIzol LS (Invitrogen, Carlsbad, CA, USA)). The RNA was retrotranscribed to cDNA with SuperScript II (Invitrogen) and random primers. Two different PCR protocols were used on the same samples: an RT nested PCR[14] and a real-time PCR with Taqman probe.[15]

Two pairs of primers (CHIKV 10264F/CHIKV 11300R and CHIKV 10564F/CHIKV 11081R)[16] were used to

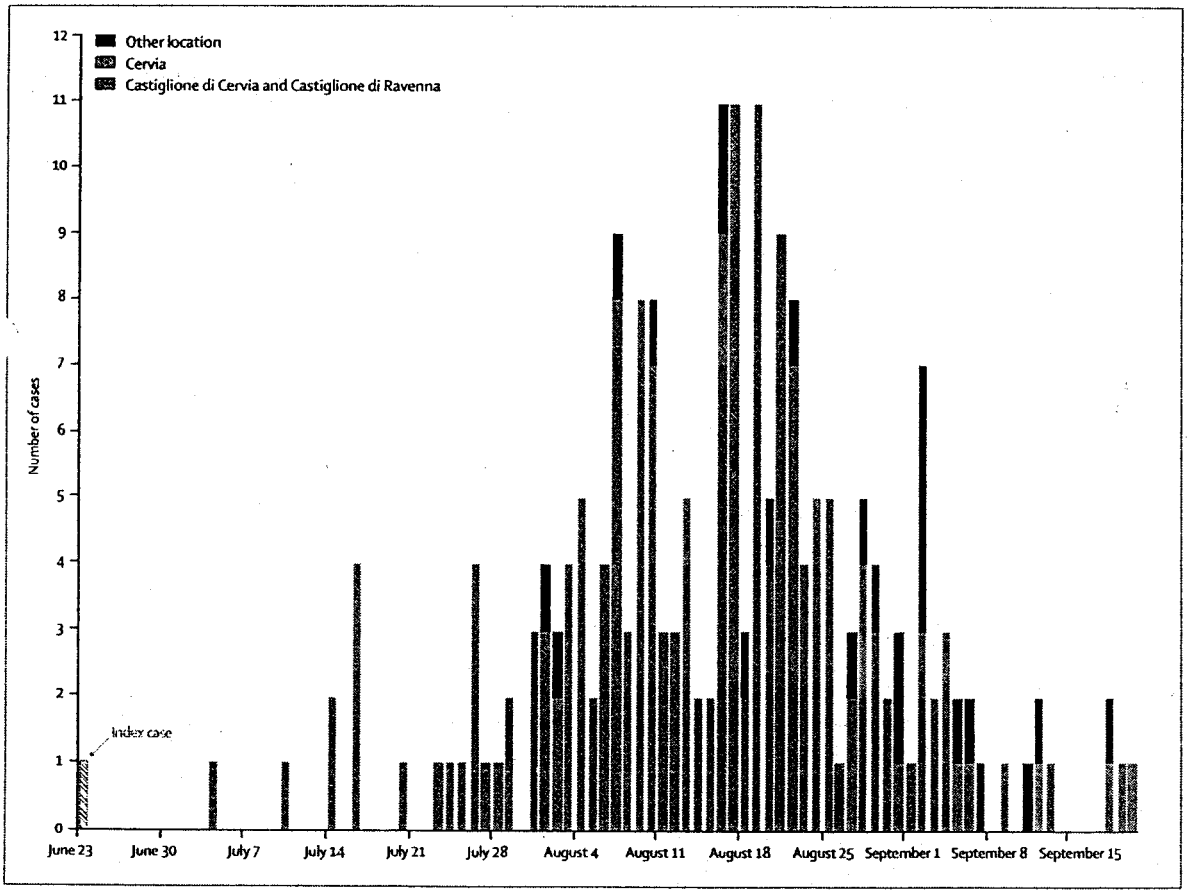

Figure 1: **Epidemic curve**
Distribution of dates of onset of symptoms for CHIKV cases by presumed place of infection (ie, Castiglione di Cervia and Castiglione di Ravenna, Cervia, or other/unknown location).

amplify part of the E1 gene directly from the extracted RNA from RT-PCR-positive samples. Nucleotide sequences were assembled with BioEdit software (version 7·0·9·0) and were aligned with Clustal W software (version 1.6). Phylogenetic analysis based on the available partial E1 gene sequences of CHIKV and tree reconstructions were done with MEGA version 4. For the construction of phylogenetic trees, the neighbour-joining algorithm and the Kimura two-parameter distance model were used. The reliability of the analysis was assessed by a bootstrap test with 1000 replications.

Statistical analysis

In the present analysis, we considered those cases identified between July 4 and Sept 27, 2007. The dates of the onset of symptoms of the cases were plotted to fit the epidemic curve. The frequency distribution of the cases' main characteristics and their signs and symptoms were calculated. Attack rates (both overall and stratified by age and sex) were calculated for the two villages that were affected. Risk ratios (RR) and their 95% CI were also estimated. Age-adjusted attack rates were also calculated separately by sex. The origin and spread of CHIKV cases in the two initially affected villages were mapped. For each case, the address of the individual and the date of the onset of symptoms were entered into Microsoft Access 2003 and linked to the locations on georeferenced maps in the geographic information system (ArcView 8·3, ESRI, Redlands, CA, USA).

Role of the funding source

There was no funding source for this study. All authors had full access to all the data. The corresponding author had final responsibility for the decision to submit for publication.

Results

205 cases of CHIKV infection occurred between July 4 to Sept 27, 2007, in Ravenna (figure 1). There were several waves of cases, with the number peaking in the third week of August. Up to the time of this peak, most cases had occurred in Castiglione di Cervia and Castiglione di Ravenna. Afterwards, and after the first mosquito control measures in the area that was mainly affected had been implemented (on Aug 18), a new wave of cases was observed, most of which occurred outside the two villages.

The distribution of cases by age, sex, residence, and place where the infection was presumably acquired is shown in table 1. The median age was 60 (range 1–95) years; 58 (1–92) years for male cases and 62 (3–95) years for female cases. Most patients reported that they lived in or had visited one of the two villages. The others were scattered throughout the province, although a cluster of 13 cases due to local transmission was reported in Cervia, a town of 8606 inhabitants located about 9 km from the villages. This cluster occurred in a restricted area of the town, near a public gathering place frequented by people coming from—or who had visited—Castiglione di Cervia and who had developed the disease.

	Number of cases (%)
Age (years)	
0–19	12 (6%)
20–39	26 (13%)
40–59	62 (30%)
60–70	78 (38%)
≥80	27 (13%)
Sex	
Male	99 (48%)
Female	106 (52%)
Presumed place of infection	
Castiglione di Cervia or Castiglione di Ravenna	171 (83%)
Cervia	13 (6%)
Other/unknown	21 (10%)
Classification of cases	
Laboratory confirmed	175 (85%)
Clinically defined (untested)	30 (15%)

Table 1: Demographic characteristics of the 205 individuals infected with CHIKV

The first identified case, a man of Indian origin living in Castiglione di Cervia, reported that he had not been abroad during the previous year. However, a relative of his, who had arrived in Italy on June 21 from Kerala, India (an area affected by the CHIKV epidemic), visited the man and became feverish on the afternoon of June 23, when he was in Castiglione di Cervia. A serum sample that had been collected in early September from this man, who was assumed to be the index case, showed high antibody titres against CHIKV (>1:1280). This individual was excluded from the further data analyses.

The spatial-temporal spread of CHIKV in the primarily affected area and the rest of the province is shown in figure 2. After the first cases, which occurred in Castiglione di Cervia, the infection spread both by contiguity, as an expansion of the primary cluster (figure 2 A, B, and C), and by jumping from place to place in both villages, with cases developing more than 2 km away from the primary cluster (figure 2 B and C). Sporadic cases and clusters occurring outside the villages are shown in figure 2 D.

The attack rate was 5·4% in Castiglione di Cervia (115 resident cases out of 2134 inhabitants) and 2·5% in Castiglione di Ravenna (46/1834). The attack rate did not differ between female and male individuals (4·5% of 81 females *vs* 4·0% of 80 males; RR 1·13, 95% CI 0·81–1·57). The rate of attack increased with age: 1·6% of 27 people under 40 years of age, 4·5% of 52 individuals aged 40–59 years, 7·0% of 57 aged 60–79 years, and 8·8% of 25 aged 80 years or older were affected (RR 2·78, 95% CI 1·75–4·39 for the 40–59 years age-group; 4·21,

Figure 2: **Geographical origin and spatial-temporal diffusion of CHIKV cases**
Number of cases in Castiglione di Cervia and Castiglione di Ravenna between days 0–15 (A), between days 0–45 (B), cumulatively (C), and in the province of Ravenna (D).

2·86–6·61 for the 60–79 years age-group; and 5·20, 3·08–8·83 for those aged 80 years or more, all relative to the under 40 years age-group; χ^2 for trend p<0·0001). There was no difference in attack rate between those aged 0–19 years and those aged 20–39 years (1·6% [10/631 individuals] *vs* 1·6% [17/1082]). The age-adjusted attack rates for male and female individuals were much the same (4·2% *vs* 4·1%).

The frequency of clinical symptoms is shown in table 2. All patients presented with high fever (median maximum temperature 39·5°C, 25–75th percentile 39–39·8°C), and most of them had pain in multiple joints. About half the cases developed skin rash, in some cases with itching. Clinical disease was mild and self-limiting in most cases. One 83-year-old man died, although this man had severe underlying conditions.

Laboratory confirmation was obtained for 175 cases: 32 were PCR-positive only; 135 were haemagglutination inhibition-positive only; and eight were positive for both PCR and haemagglutination inhibition. The median time between the onset of symptoms and obtaining positive results was 2 days for PCR (maximum 7 days) and 15 days for haemagglutination inhibition. 30 cases who met the clinical and epidemiological criteria

	Number of cases (%)
Fever*	205 (100%)
Joint paint	199 (97%)
Fatigue	190 (93%)
Skin rash	106 (52%)
Headache	105 (51%)
Muscle pain	94 (46%)
Diarrhoea	48 (23%)
Itching	42 (20%)
Vomiting	40 (19%)
Photophobia	31 (15%)
Conjunctivitis	7 (3%)

*Mandatory in the case definition. †Not mandatory if diagnosis is laboratory confirmed.

Table 2: **Distribution of symptoms**

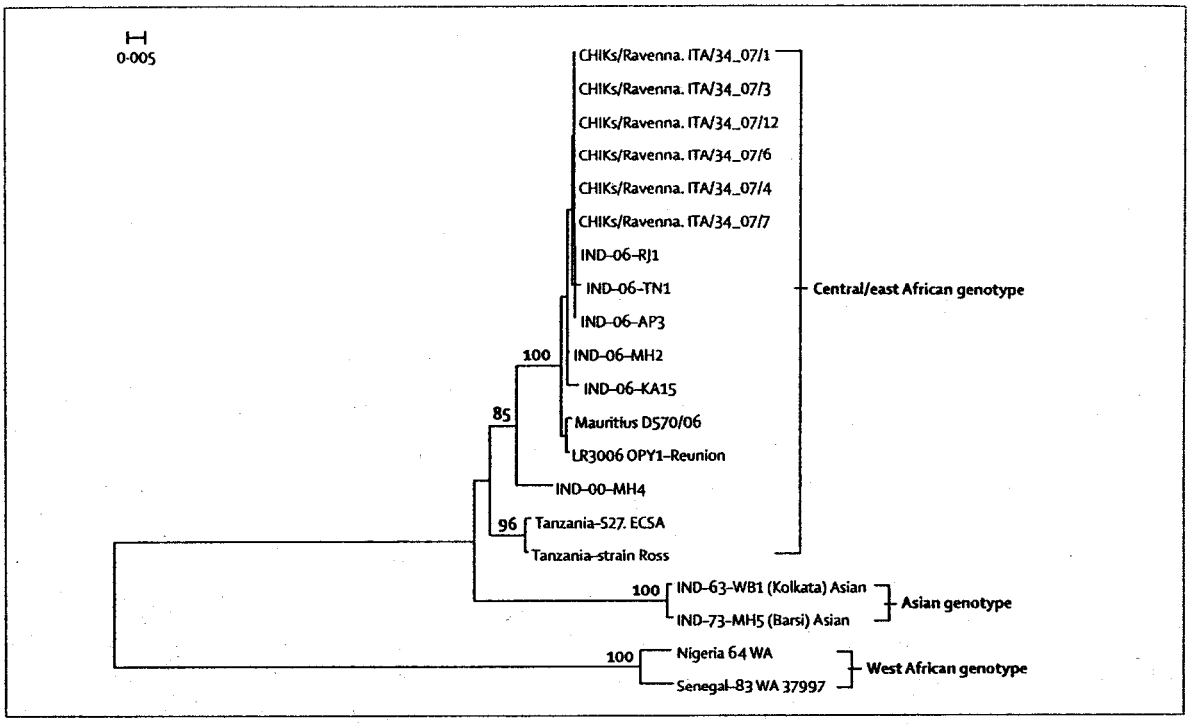

Figure 3: **Phylogenetic analysis of the partial nucleotide sequence (1011 nucleotides) of the E1 gene of CHIKV strains identified in Italy and in different parts of the world**

remained untested because no blood sample was available or because it was inadequate for testing (table 1).

CHIKV sequences were detected by PCR in *A albopictus* mosquitoes captured during the outbreak. Positive results were obtained from pools of 125 mosquitoes from Castiglione di Ravenna and and 90 from Castiglione di Cervia. The results of the phylogenetic analysis are shown in figure 3. Human and mosquito strains clustered with Indian strains, and they contained a change (Ala226Val) in the membrane fusion glycoprotein E1 that had also been found in the Indian Ocean variant of the African genotype of CHIKV.

Discussion

This outbreak of CHIKV infection, outside a tropical country, was probably begun by a man from India, who developed a febrile syndrome 2 days after his arrival in Italy. He had high titres of antibodies against CHIKV at the time of examination (early September) and was probably highly viraemic when visiting his relatives (late June) in the village where the epidemic began. The phylogenetic analysis showed that the strain that caused this outbreak was similar to the strains detected on the Indian subcontinent[5] and that it contained the same mutation found in a variant in the Indian Ocean islands,[7,17] which is thought to be better adapted to *A albopictus* than are other variants. The hypothesis that this variant has a high virus-vector fitness seems to be confirmed by both the successful introduction and rapid spread of the infection from one infected human host and by the further occurrence of other smaller clusters in different localities in the same province yet located several kilometres from the two villages initially affected.

Samples of *A albopictus* mosquitoes from the villages were found to be positive for CHIKV sequences. The high density of the vector at the time of arrival of the index case, as anecdotally reported by villagers, was probably a major determinant of the outbreak. Actually, the population of *A albopictus* was already well established in scattered foci in Ravenna province (an average of >80% positive ovitraps), but had only recently enlarged its peripheral area to include these villages, which might explain the high vector density (ie, before control measures had been implemented). The presence of *A albopictus* in Italy is not surprising. The mosquito was first documented in Genoa (northwestern Italy) in 1990,[18] and the presence of a breeding population was first reported near Padua (northeastern Italy) in 1991.[19] The source of infestation was identified as a warehouse of a tyre retreading company that had imported used tyres infested with mosquito eggs from Georgia, USA.[20] Unfortunately, despite efforts made to control the spread of *A albopictus* mosquitoes, they rapidly colonised almost the entire country,[21,22] showing a high degree of fitness.

The peak of the outbreak occurred during the third week of August, more than 6 weeks after the onset of symptoms in the first locally acquired case, and 8–9 weeks after the onset of symptoms in the presumed

index case. The occurrence of new cases in the initially affected area started to decrease a few days after vector control measures were first implemented. The infection seemed to spread both by contiguity within the initially affected villages and by jumping from place to place within and from the initial outbreak area to the other locations. A small cluster, caused by local transmission, was reported in the town of Cervia, where the infection was probably introduced through population movement from Castiglione, although passive transport of infected mosquitoes cannot be ruled out completely.

The attack rate in Castiglione di Cervia—the most affected village—was 5·4%, much lower than the 34% reported in La Réunion.[4] This difference might be due to early intervention in Italy, although the role of different background vector density or climate-dependent vector behaviour cannot be excluded. Moreover, we cannot rule out under-reporting, which could have occurred if our surveillance system had a low sensitivity in the first month or if there was an excess of asymptomatic cases compared with those found in La Réunion.[23] The attack rates by sex and age, calculated for Castiglione di Cervia and Castiglione di Ravenna, were stably low for people under 40 years of age but tended to increase for older ages, with the highest rates in the oldest group. Whether this trend was due to behavioural factors leading to differential exposure to mosquitoes or to biological factors, implying a different host response with a different proportion of asymptomatic cases, needs to be investigated further.

The clinical course of the disease was fairly mild. The case-fatality rate was less than 0·5%, consistent with the rate of one death per 1000 clinical cases reported in La Réunion.[4] Almost all patients reported joint pain, which was often severe and persistent, and which seems to be strongly indicative of CHIKV disease. Similar findings were reported in La Réunion,[24] whereas a lower proportion of cases with joint pain (78%) was found in Malaysia in 1998.[25] About half the patients presented with skin rash, similar to previous findings.[2]

85% of the cases were confirmed by either serology or PCR. No viral sequences were detected in 31 samples collected more than 7 days after the onset of symptoms, suggesting that the viraemic phase is fairly short, as found in previous reports.[26]

Measures for controlling the population of *A albopictus* were implemented in all areas where cases were reported, beginning on Aug 18. These measures included the use of fast-acting insecticides (synergised pyrethrins) for 3 days consecutively, applied with a truck-mounted atomiser in public spaces 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 done to eliminate breeding places, and community participation was encouraged. For each suspected case of infection, these control measures were done within a radius of 100 m of the individual's residence; for clusters, the control measures were done within a 300-m radius of the most external case. Since Sept 27, 2007—the date at which the present analysis was censored—sporadic cases have continued to occur in Ravenna; two small clusters outside Ravenna (in Cesena and Rimini) have also been identified. Whether transovarial transmission of CHIKV might result in a reappearance of the infection in spring, 2008, is being considered carefully.

The occurrence of an outbreak of CHIKV infection in a country with a temperate climate emphasises that the predicted globalisation of human beings and vectors[4,7] has become a reality. To promptly identify new potential threats that were previously restricted to tropical areas, clinical and diagnostic capacities have to be developed in countries with a temperate climate and in which vectors of exotic diseases already circulate.

Contributors

GR was responsible for the clinical and epidemiological investigation and for writing the manuscript. LN was responsible for laboratory diagnosis and contributed to writing the manuscript. CF, FM, and MGC did laboratory tests on human and mosquito samples, and phylogenetic analysis; PC and MD identified viral sequences in the mosquitoes. MP developed the PCR used in this investigation and contributed to writing the manuscript. RR, GM, and PA were responsible for the entomological investigation and contributed to writing the manuscript. ACF supervised the field activities that were implemented by RA and GS, who also contributed to data analysis. SB was responsible for data management and analysis. AC supervised and coordinated all of the activities and revised the manuscript.

CHIKV study group

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Conflict of interest statement

We declare that we have no conflict of interest.

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