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AN OUTBREAK OF DENGUE VIRUS SEROTYPE 1 INFECTION IN CIXI, NINGBO, PEOPLE'S REPUBLIC OF CHINA, 2004, ASSOCIATED WITH A TRAVELER FROM THAILAND AND HIGH DENSITY OF *AEDES ALBOPICTUS*

GUOZHANG XU, HONGJUN DONG, NANFENG SHI, SHIJIAN LIU, AIMING ZHOU, ZHIHUA CHENG, GUOHUA CHEN, JIANYI LIU, TING FANG, HONGWEI ZHANG, CHUNYING GU, XIAOJIE TAN, JIANJIE YE, SHUYUN XIE, AND GUANGWEN CAO*

Department of Infectious Diseases, Center of Disease Prevention and Control of Ningbo City, Ningbo, Zhejiang, People's Republic of China; Department of Infectious Diseases, Center of Disease Prevention and Control of Cixi County, Cixi, Ningbo, Zhejiang, People's Republic of China; Department of Epidemiology, Second Military Medical University, Shanghai, People's Republic of China; Department of Infectious Diseases, Center of Disease Prevention and Control of Zhejiang Province, Hangzhou, Zhejiang, People's Republic of China

Abstract. Autochthonous dengue infections have not been reported in Ningbo, People's Republic of China since 1929. In August–October 2004, an outbreak of dengue fever was confirmed in Xiaolin, Cixi, Ningbo. Of 83 cases reported, 68 were laboratory confirmed. Fifty-three percent (34 of 64) of the cases had IgM antibodies to dengue virus. Dengue virus serotype-1 was isolated from two cases. The outbreak was linked to a traveler who returned from Thailand. Phylogenetic analysis showed that the Ningbo isolate was closely associated to strains from Thailand. Prevalence of dengue-specific IgG in asymptomatic residents was significantly higher in the epidemic-stricken area than in a control area. High density of *Aedes albopictus*, which resulted from waterlogging caused by Typhoon Rananim and lifestyle of local residents, was responsible for rapid spread of the virus. Eradication of mosquito infestation might interrupt transmission. This outbreak underscores the importance of maintaining surveillance and control of potential vectors for the control of emerging infectious diseases.

INTRODUCTION

Dengue is a mosquito (*Aedes aegyti* and *Ae. albopictus*)–transmitted, acute viral disease caused by any of four antigenically distinct serotypes of dengue virus (DEN-1, DEN-2, DEN-3, or DEN-4). Dengue is endemic in most tropical and subtropical areas of the world, with an estimated annual occurrence of 100 million cases of dengue fever (DF) and 250,000 cases of dengue hemorrhagic fever (DHF). Dengue fever has been reported in more than 100 countries, with 2.5 billion people living in areas where dengue is endemic.[1] Demographic and societal changes such as population growth, urbanization, and modern transportation greatly contributed to the increased incidence and geographic spread of dengue activity. The World Health Organization (WHO) classifies dengue as a major international public health concern because of the expanding geographic distribution of virus and mosquito vector, the increased frequency of epidemics, the cocirculation of multiple virus serotypes, and the emergence of DHF in new areas.[2] International travelers may spread dengue virus infection from DF-endemic areas to new regions.[3]

In Mainland China, an epidemic of dengue caused by DEN-1 and DEN-4 was reported in Shiwan Town, Foshan City, Guangdong Province, in 1978–1979.[4] In 1980, a dengue outbreak occurred on Hainan Island. DEN-1 and DEN-3 were isolated from sera of acute-phase patients and pools of adult *Ae. aegypti*.[5] In 1985–1986, the first epidemic of DHF in China occurred on Hainan Island. The morbidity rate was 1,913 per 100,000 residents, with a case fatality rate of 0.25%. DEN-2 was isolated from sera of acute-phase patients.[6] Since the 1990s, dengue epidemics have frequently occurred in Guangdong, Guangxi, and Hainan provinces. *Aedes aegypti* was the vector in coastal areas, and *Ae. albopictus* was the vector in inland regions of China. The reemergence of dengue in China usually resulted from the introduction of the infection by travelers and refugees from areas of southeast Asia where dengue was endemic.[7]

In Ningbo, Zhejiang Province of China, no dengue epidemic had been recorded in the past 75 years.[8] In early September 2004, families-based clustering cases with acute febrile illness from seven adjacent rural villages of Xiaolin Town, Cixi, Ningbo drew serious attention in two public hospitals in the metropolitan area of Ningbo. The number of cases kept increasing and caused fear in local communities. Our epidemiologic team conducted the investigation on this undifferentiated acute febrile disease. A dengue outbreak was then identified based on the basis of epidemiologic, clinical, and laboratory examinations that met the case definition.[9] Efforts to temporarily eradicate mosquito infestation contributed to the cessation of transmission.

METHODS

The participants in this study were enrolled in protocols reviewed and approved by the appropriate institutional review boards. Informed consent was not obtained for participation in this study. However, the identity of all participants was kept confidential.

Characterization of clinical illness. Clinical information was collected from those residents who were admitted to the hospitals in Ningbo between September and October 2004. A standardized questionnaire was used to record the presence or absence of fever, chills, fatigue, rash, myalgia, arthralgia, joint pain, anorexia, cough, and gum bleeding; and the presence or absence of physical signs elicited on examination, including cervical lymphadenopathy, flushing, petechiae, and suffused eyes. Results of laboratory tests performed as part of routine clinical care were recorded from case-sheets, includ-

* Address correspondence to Guangwen Cao, Department of Epidemiology, Second Military Medical University, 800 Xiang-yin Road, Shanghai 200433, People's Republic of China. E-mail: gcao@smmu.edu.cn

ing hematologic and biochemical examination, bacterial cultures of blood, and serologic tests for antibodies to dengue, rickettsioses, typhoparatyphoid, and Japanese encephalitis. Demographic, geographic, and temporal data were collected from residents with febrile disease from the affected villages. An index case and the cases with the disease in August 2004 were retrospectively traced on the basis of clinical, laboratory, and epidemiologic data.

IgM-capture enzyme-linked immunosorbent assay (ELISA). Serum specimens from acute-phase patients were obtained and stored at -20°C. A dengue IgM-capture ELISA (Zhongshan Bioengineering Inc., Guangdong, People's Republic of China) was performed according to the manufacturer's instructions.

Virus isolation and RNA extraction. Sera of acute febrile cases were obtained within six days after the onset of disease. Virus isolation was performed by adding the serum samples into the *Ae. albopictus* cell line C6/36, which was grown in plastic tissue culture flasks (T-25). Dulbecco's modified Eagle's medium (DMEM) (Gibco-BRL, Gaithersburg, MD) supplemented with 10% fetal calf serum (FCS) (Gibco-BRL) was used. The cell monolayer was incubated with 200 μL of serum sample at 28°C in an atmosphere of 5% CO_2 for one hour before 2 mL of DMEM supplemented with 2% FCS was added. The cultures were incubated for seven days and observed daily for cytopathic effects (CPEs). At the end of the seven-day incubation, the supernatant was harvested and clarified by centrifugation at 2,500 rpm before incubation onto a fresh cell monolayer. The RNA was extracted from 200 μL of sera from 12 acute-phase febrile patients and from viral culture supernatants by using the RNAeasy Mini Kit (Qiagen, Hilden, Germany).

Reverse transcription–polymerase chain reaction (RT-PCR) and sequencing. An RT-PCR developed by Lanciotti and others[10] was used for the detection and identification of dengue viral RNA by One Step RNA PCR Kit (TaKaRa Biotech Co., Dalian, People's Republic of China). The RT-PCR products were analyzed by electrophoresis on a 2% agarose gel, stained with ethidium bromide, and visualized under ultraviolet radiation. Genomic RNA of the isolates was reverse transcribed into cDNA using random hexamer oligonucleotides with the SuperScript first-strand synthesis system (Invitrogen, Carlsbad, CA). DNA fragments of the envelope (E) gene and the overlapping DNA fragments that included the genome of the isolate were amplified by PCR with Proofstart (Qiagen) and *Taq* DNA polymerase (Promega, Madison, WI) and submitted for DNA sequencing to the Shanghai Bioengineering Center of Chinese Academy of Science (Shanghai, People's Republic of China).

Phylogenetic analysis. A total of 61 DEN-1 strains were retrieved from GenBank with different temporal and geographic origins, and named by the strain number followed by country abbreviation and year of isolation (Supplementary Table). NB01Cho4 (E gene of DQ836632) and the reference sequences were aligned using the CLUSTAL X 1.81 algorithm with default parameters. Phylogenetic trees for the entire sequence of E gene were obtained using different tree building methods either by MEGA (version 3.1) or PHYLIP (version 3.65).[11] Bootstrap analysis with 1,000 replicates was used to determine the robustness of the tree and the evolutionary relationship of DEN-1.

Entomology. Cixi (30°02′–30°24′N, 121°02′–121°42′E) has a monsoon climate. The mean annual rainfall is 1272.8 mm, and mean annual temperature 16.0°C. Xiaolin Town is in the center of Cixi. The population in Xiaolin is approximately 82,000 including 41,108 native residents and 41,000 temporary workers from other provinces. Because the dengue outbreak was confirmed on October 5, 2004, we conducted entomologic inspections in Xiaolin to collect and identify mosquito larva in all water-containing receptacles in and around the dwellings on October 6–8, 2004. Devices to trap adult mosquitoes and mosquito larva were installed in a sampling of houses. An estimate was made of infestation by *Ae. aegyti* and *Ae. albopictus* using standard larva survey: Breteau index (BI) (number of containers with immature stages per 100 houses), house index (HI) (number of houses containing immature stages per 100 houses), and container index (CI) (number of containers with immature stages per 100 containers with water). Identification of the species of the larva was carried out after the fourth development stage.

To investigate the possibility of dengue virus transovarial transmission, adult mosquitoes and immature forms of *Ae. albopictus* were collected from households and nearby ponds where residents were diagnosed with DF. After collections, mosquitoes were anesthetized on ice, identified, pooled by sex, date, and place of capture. Size of pools ranged from 5 to 50 mosquitoes. After homogenization, RT-PCR with primers D1 and D2 was used for identification of dengue virus in each pool.[10]

Comprehensive intervention to reduce mosquito density was enforced from October 8 to 15, 2004. The methods included spraying of pyrethroids and dichloro-diphenyl-trichloroethane (DDT), cleaning up all discard materials in and around the dwellings to eradicate the larva infestation, and personal protection by curtain and repellents. Control efficacy was evaluated by the density of mosquitoes that remained in a subsequent survey. Dengue cases in this area were surveyed in the summer of 2005 and 2006.

Serologic survey for antibodies to dengue virus. In March 2005, a serologic survey antibodies to dengue virus was carried out in the epidemic-stricken area (Xiaolin) and control area (other towns in Cixi without dengue cases). Cluster sampling method was used. The sera from asymptomatic residents during dengue epidemic were sampled for IgG antibody to dengue virus by an indirect immunofluorescence assay, as described.[12] The sera from the convalescent dengue patients and normal sera served as positive and negative control, respectively. An IgG titer > 1:80 was considered positive.

RESULTS

Index case confirmation and outbreak description. The index case was a 54-year-old Chinese mechanic who traveled to Thailand on May 27, 2004, and returned on July 19, 2004. On July 23, he had a fever (39°C–40°C), showed fatigue, and was admitted into a clinic in Poshan village of Xiaolin. Hematologic examination indicated thrombocytopenia (1.9×10^{10} platelets/L) and leukopenia (2.1×10^{9} cells/L). He was misdiagnosed as having a viral infection of the upper respiratory tract. After anti-inflammatory treatment, he was discharged from the hospital on August 3, 2004, and rested at home in Xiaoluyan village. On the basis of clinical and epidemiologic data and serum IgM antibody to dengue virus, his diagnosis

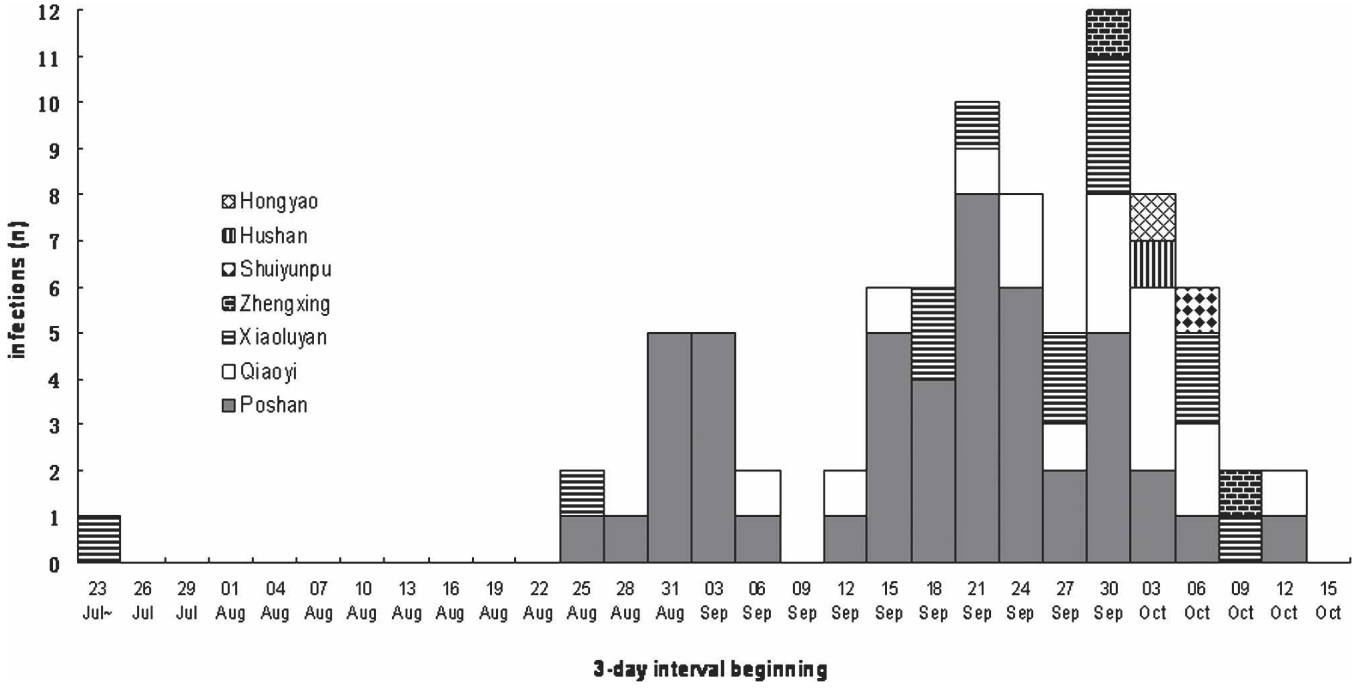

FIGURE 1. Reported dengue infections by 3-day of illness onset and villages, from July 22, 2004 to October 15, 2004, Cixi, Ningbo, People's Republic of China.

was then changed to be DF on October 5, 2004. This case was confirmed to be the index of this outbreak.

The dengue cases clustered from August 25 to October 14, 2004 (Figure 1). A total of 83 (including the index) hospitalized patients were diagnosed as clinical probable or confirmed cases by epidemiologic and clinical data.[9] Sixty-eight cases were laboratory confirmed by isolation of dengue-specific antibodies and nucleic acid in the sera.[13] Of the 83 cases (29 male and 54 female), the youngest was 7 years of age and the oldest was 76 years of age. The cases ranged in age from 20 to 55 years old accounted for 78.3% (Table 1). Most cases (49 of 83) clustered within 300 meters[2] around Poshan village. Other cases were found in the six surrounding villages. An additional 128 residents with febrile disease from July 23, 2004 to October 15, 2004 were identified by a home-to-home survey in those villages. Dengue was not confirmed in those villagers because they never visited hospitals and refused to provide serum samples for further examination.

Clinical information of hospitalized patients. A total of 83 diagnosed dengue cases showed the following laboratory findings: high fever, 100%; fatigue, 86.59%; anorexia 69.88%; rash and macular change, 62.19%; myalgia and arthralgia, 45.78%; headache, 59.04%; cervical lymphadenopathy, 5.26%; leukopenia, 88.73%; and thrombocytopenia, 73.24%.

Results of routine microbiologic examinations for other viruses and bacteria by culture and antigen detection were negative in all cases. Serologic tests for *Rickettsia typhi*, typhoparatyphoid *Salmonella*, and Japanese encephalitis virus were performed in 12 patients and all results were negative.

IgM-capture ELISA and RT-PCR. Of 64 proven cases, 34 (53.2%) had IgM antibodies to DEN-1 2–55 days after the onset of disease. DEN-1 RNA was identified in sera of five patients by RT-PCR with consensus primers D1 and D2 within six days after the onset of disease (Figure 2). Seven days after the onset of fever, viral RNA could not be detected. All RT-PCR-positive serum samples were negative for IgM antibodies to dengue virus.

Viral isolation and nucleotide sequencing. Dengue virus was isolated from 2 of the 5 cases with positive RT-PCR results. Microscopic examination of the cell culture inoculated with serum samples showed CPE with changes in the monolayer such as formation of vacuoles and syncitial cells at the second passages (Figure 3A and B). RNA isolated from supernatants of cell culture with or without CPE was analyzed by the RT-PCR assay. The correctly sized DNA products (511 basepairs) were obtained after amplification with consensus primers D1 and D2 (Figure 3C). A DNA fragment

TABLE 1
Age and sex of total and laboratory-confirmed dengue cases

Age range (years)	Total dengue cases		Laboratory confirmed cases no. (men, women)
	No. (men, women)	%	
0–4	0 (0, 0)	0	0 (0, 0)
5–9	4 (3, 1)	4.8	3 (2, 1)
10–14	0 (0, 0)	0	0 (0, 0)
15–19	6 (2, 4)	7.2	6 (2.4)
20–24	6 (1, 5)	7.2	5 (1, 4)
25–30	6 (4, 2)	7.2	5 (4, 1)
30–34	12 (4, 8)	14.5	9 (4, 5)
35–39	11 (5, 6)	13.3	10 (5, 5)
40–44	8 (2, 6)	9.6	8 (2, 6)
45–49	3 (2, 1)	3.6	2 (1, 1)
50–54	9 (2, 7)	10.8	7 (2, 5)
55–59	10 (1, 9)	12.0	8 (1, 7)
60–64	4 (1, 3)	4.8	3 (0, 3)
65–69	2 (0, 2)	2.4	1 (0, 1)
70–75	1 (1, 0)	1.2	1 (1, 0)
75	1 (1, 0)	1.2	1 (1, 0)
Total	83 (29, 54)	99.8	68 (26, 42)

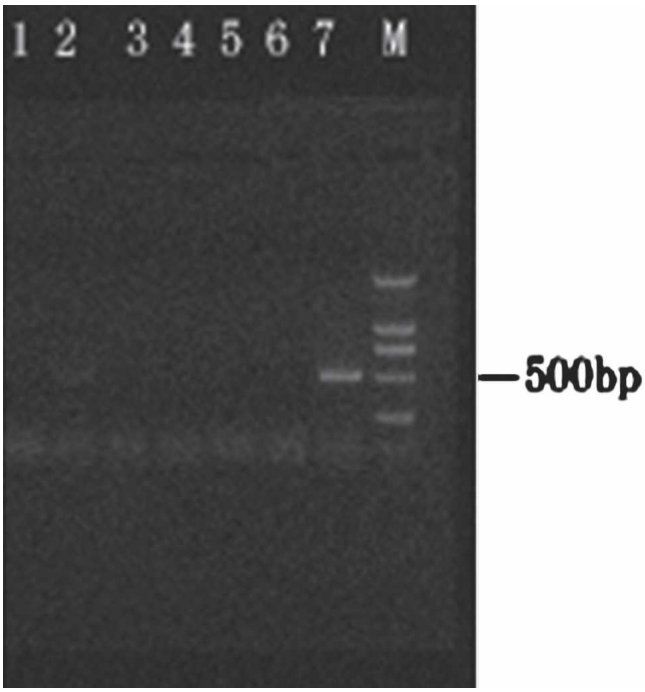

FIGURE 2. Dengue viral RNA identified in sera of patients by reverse transcription–polymerase chain reaction with consensus primers D1 and D2 and generated 511-basepair (bp) fragments within 6 days after onset of disease. Lane 1, negative control; lanes 2–7, sera from patients; lanes 2 and 7, positive sera; lane M, DNA marker.

(482 basepairs), which was the expected size for the DEN-1 target region of the genome, was exclusively obtained after amplification with serotype-specific primer pairs and supernatants of cultures with sera of both patients (Figure 3D). The E gene fragments of the viruses were amplified. Analysis of amplicon DNA sequences showed 100% homology with E gene of viruses from the matched cases. Partial sequence (5,286 basepairs) of the viral genome from patient A was sequenced and submitted to GenBank with accession no. DQ836632.

Phylogenetic analysis. Maximum likelihood, maximum parsimony, and neighbor-joining (NJ) methods were used to create the phylogenetic tree. Although the three methods yielded a tree with similar clustering, the branching order of the basal nodes differed between methods. From the phylogenetic tree created by NJ method, the Ningbo isolate and six strains from Thailand were clearly grouped together in a well-supported distinct cluster and assigned to genotype 1 (Figure 4).[14] Those strains were 0261Th01 (AY732419), 0102Th01 (AY732392), 0499Th01 (AY732389), and 0075Th02 (AY732398), which were obtained in Thailand in 2001–2002, and Hu65Th01 (AB111072) and Hu38Th02 (AB11078), which were obtained in Japan in 2001–2002 from imported cases from Thailand. This analysis suggested that the Ningbo isolate was from Thailand.

Entomologic examination. Mosquitoes were inspected and morphologically identified in each of four villages with dengue cases. No *Ae. aegypti* was found. *Aedes albopictus*, *Anopheles hyrcanus sinensis*, and *Culex quinquefasciatus* accounted for 20.31%, 41.2%, and 37.5% of the adult mosquitoes, respectively. *Aedes albopictus* is a well-known dengue vector.[1–6] *Anopheles* and *Culex* mosquitoes are resistant to dengue infection.[15] *Aedes albopictus* was most frequently found in the inner side of the larva containers surrounding trash and grass. Density of *Ae. albopictus* was extremely high in the first inspection. The average BI, CI, and HI values for larva of *Ae. albopictus* were 260.55, 55.05 and 77.78, respectively. The RT-PCR with consensus primers D1 and D2 was used for detection of dengue viral RNA from homogenized female *Ae. albopictus*. The dengue-specific 511-basepair fragment was amplified from a pool of 10 mosquitoes. After the comprehensive interventions, BI for larva of *Ae. albopictus* was less than 5 (Table 2). In May 2005, we collected mosquito larva from households and ponds around dwellings of dengue cases. After growing in the laboratory, *Ae. albopictus* were homogenized in pools of 5–50 mosquitoes. The RT-PCR and subsequent nested RT-PCR were used for identification of dengue RNA.[10] All reactions showed negative results.

Serologic survey for antibody to dengue virus. Based on availability of participants, 520 asymptomatic residents from the epidemic-stricken area and 187 residents from the control were tested in the serologic survey. Of 520 asymptomatic residents tested, 35 (6.7%) including 7 men and 28 women had IgG antibody to dengue virus. Of the 187 controls tested, only one man (0.5%) had IgG antibody to dengue virus. In the epidemic-stricken area, 7 (4.0%) of 174 men and 28 (8.1%) of 346 women had IgG antibody to dengue virus (Table 3). The number of men was less than the number of women because most men left their hometown for job opportunities in large cities during the survey.

DISCUSSION

This report describes the first outbreak of DF in Ningbo area, Zhejiang, China since 1929. Understanding the factors that contributed to the re-emergence of dengue after such a prolonged absence will help public health authorities develop practicable prevention and control strategies.

A total of 83 hospitalized cases were diagnosed as having DF according to WHO criteria. Of the 64 cases tested, 34 (53.2%) had IgM antibody to DEN-1. DEN-1 RNA was identified in sera of five patients by RT-PCR. The remaining samples were negative for an etiologic agent, probably because of single sampling in the acute phase of the disease.

To trace the source of infection, an epidemiologic study, serologic examination, and phylogenetic analysis were performed in the cohort of infected persons. Serologic and epidemiologic data strongly suggested that the dengue outbreak was directly linked to the person who returned from Thailand. Antibody tests and a serotype-specific PCR confirmed that DEN-1 was the unique etiologic agent in this outbreak. Phylogenetic analysis showed that the Ningbo isolate and DEN-1 strains from Thailand were grouped together in a well-supported distinct cluster of DEN-1 (Figure 3). Dengue epidemics caused by DEN-1 were recently reported in other areas.[16–20] Dengue serotype 1 infection has also been reported in Guangdong and Hainan Provinces, China.[5–7] However, epidemiologic and phylogenetic analysis did not indicate that the DEN-1 strain was from those areas.

The most important prerequisite for this outbreak was the high density of *Ae. albopictus*, which might have resulted from local climate and lifestyle factors of local residents. The climate in Xiaolin is generally warm and humid. On August 12, 2004, Typhoon Rananim hit the east coast of Zhejiang. In

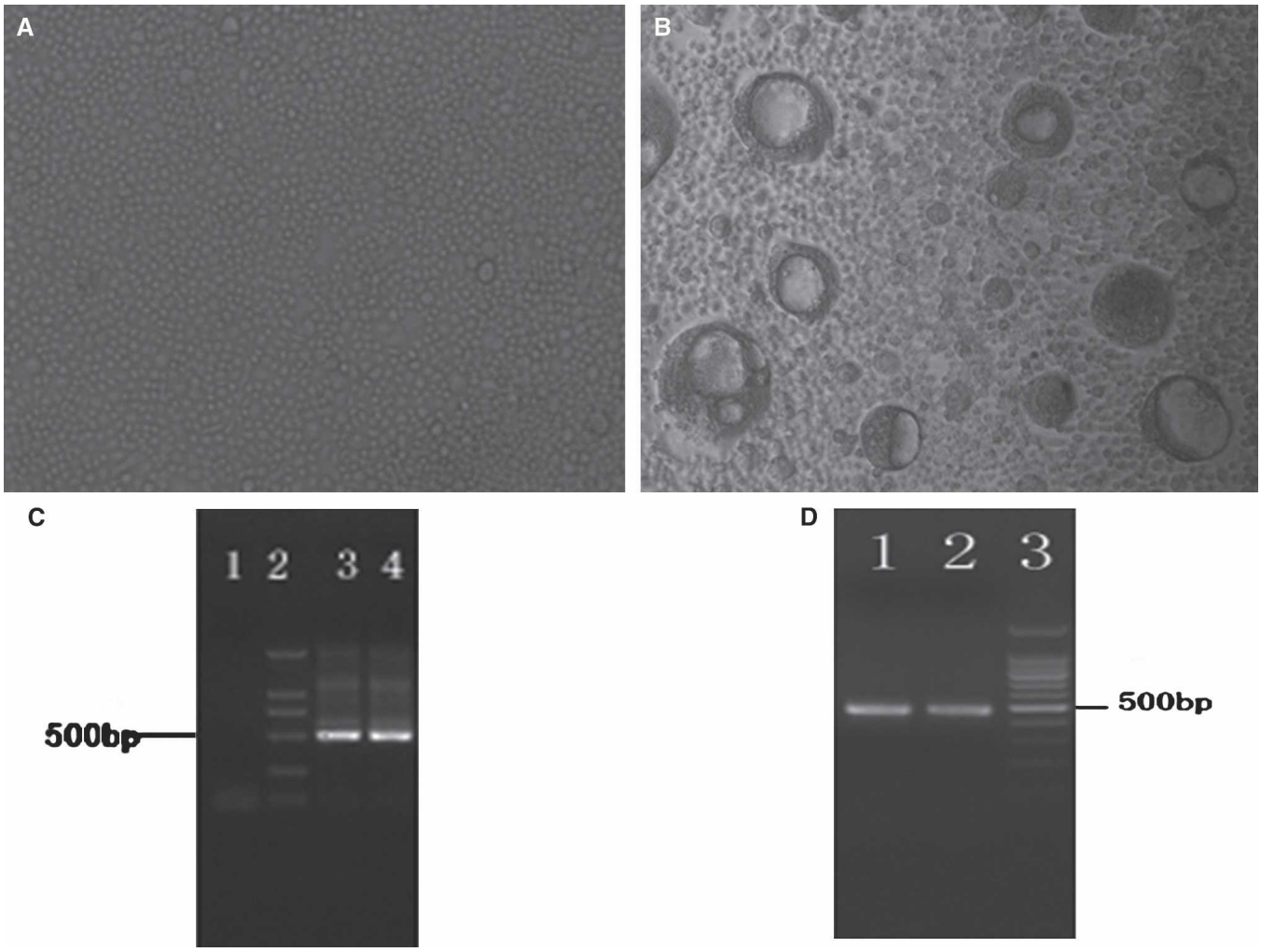

FIGURE 3. Dengue virus isolated from two of five cases positive for dengue viral RNA. **A**, C6/36 cell monolayer inoculated with control serum (original magnification × 150). **B**, Cytopathic effect (CPE) in monolayer cultured with patient serum at second passage (original magnification × 150). **C**, RNA isolated from supernatants of cell culture with CPE by reverse transcription–polymerase chain reaction. The DNA product (511-basepair [bp] fragment) was obtained after amplification with consensus primers D1 and D2. Lane 1, parental C6/36 control; lane 2, DNA marker; lanes lane 3 and 4, supernatants from cells cultured with sera of two patients. **D**, DNA fragment (482 bp) for dengue 1 virus amplified with the serotype-specific primer pairs. Lanes 1 and 2, supernatants of culture with sera of two patients; lane 3, DNA marker.

the Ningbo area, the typhoon resulted in a major storm lasted a week. As a result of the storm, tides increased and held back water flow from inland areas to the sea, which resulted in waterlogged areas. The density of mosquitoes increased to new levels in days. In Xiaolin, the economy, mainly family-owned shoe manufacturing, was highly developed, but environmental sanitation was not good. In entomologic surveys, we found that the CI, BI, and HI were extremely high (Table 2). In and around the dwellings, disposable rubber, water tanks, vats, jar-shaped vessels, tires, and earthenware containers were piled up. Sinks, natural receptacles, and grass were frequently found around dwellings in which dengue cases lived. In addition, the residents were accustomed to collecting rain water in tanks, usually 3–5 water tanks per family, for household use in case of insufficient tape water. These factors facilitated mosquito infestation. DEN-1 RNA was also found in a homogenized pool of female *Ae. albopictus* from households of dengue cases. A high density of mosquitoes contributed to the outbreak of DF after introduction of dengue virus by the traveler.

In the absence of an approved vaccine, control of the mosquito vector is the only effective preventive measure. Although the mosquito infection rate was higher during the dengue epidemic than after the epidemic period, *Ae. albopictus* can serve as a maintenance vector of dengue in rural areas of dengue-endemic countries.[21,22] Transient use of pyrethroids and DDT and cleaning up all discarded material in and around the houses to eradicate mosquito breeding in household water were successful. After mosquito control, the BI for larva of *Ae. albopictus* was less than 5 (Table 3). The reason why the index of mosquito density continued to decrease might be eradication of larva infestation and cold weather. In May 2005, dengue viral RNA could not be found in the pools of *Ae. albopictus* collected from households and ponds around dwellings of dengue cases. No new dengue case in the epidemic-stricken area was found from November 2004 to November 2006. These data indicate the cessation of dengue virus transovarial transmission.

Ningbo is not in an area endemic for dengue. A serologic survey for IgG antibody to the four serotypes dengue viruses

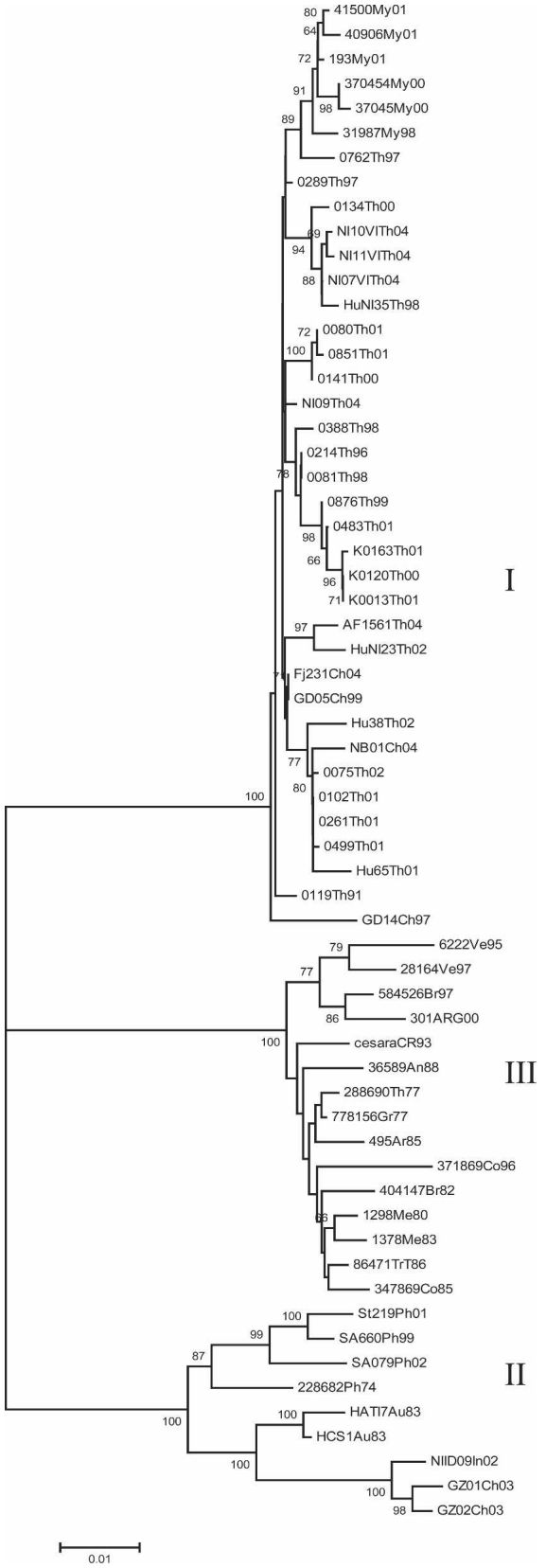

FIGURE 4. Neighbor-joining tree showing phylogenetic relationships among 62 strains of dengue 1 virus. Analysis was based on nucleotide sequences of the 1,485-basepair envelope gene of the Ningbo isolate (NB01Cho4) and the 61 DEN-1 strains obtained from GenBank. Sequences are named by the strain number, followed by country abbreviation and year of isolation.

TABLE 2

Index of larva of *Aedes albopictus* before and after the intervention*

Village	Inspection date, 2004	No. of houses inspected	Index of larva of *Ae. albopictus*		
			BI	CI	HI
Poshan	Oct 6	80	326.3	65.3	68.1
	Oct 9	62	4.0	50.0	10.0
	Oct 19	81	4.0	4.0	2.0
	Oct 27	78	1.7	1.1	1.7
Zhenxing	Oct 8	60	261.3	56.4	90.7
	Oct 20	69	16.7	17.3	13.0
	Oct 27	78	3.3	5.6	3.3
Xiaoluyan	Oct 9	62	193.6	42.2	62.0
	Oct 20	89	4.0	4.6	4.0
	Oct 28	75	2.0	2.0	2.0
Qiaoyi	Oct 8	60	261.0	56.3	89.1
	Oct 19	79	18.0	29.0	16.0
	Oct 28	80	3.3	4.3	3.3

* BI = Breteau index; CI = container index; HI = house index.

showed that the background level of IgG antibody was low in the non-epidemic area of Cixi. The percentage of asymptomatic residents with IgG antibody to dengue virus in the epidemic-stricken area was 6.7%, which was significantly higher than that in the non-epidemic area (Table 3). This finding indicated that dengue virus infection was underestimated. The true incidence of imported dengue infection must be higher because dengue may often go undiagnosed in areas where the virus is not endemic.[23,24]

Because laboratory-based etiologic diagnosis of dengue is often unavailable at the beginning of a disease outbreak, a clinical diagnosis is initially made on the basis of clinical manifestations, laboratory features, and travel experience. Comprehensive analysis of clinical, serologic, epidemiologic, and entomologic data should be an efficient way to quickly identify dengue in febrile cases in the rainy summer season. A dengue surveillance system was therefore established in all medical facilities in the Ningbo area at the end of 2004. In the summer of 2005, two introduced dengue cases from southeast Asia were quickly identified in a metropolitan hospital and isolated in a designated hospital in Ningbo. No secondary dengue cases were found.

Our investigation has several limitations. First, most of the dengue cases described were in hospitalized native residents. Most of non-native employees working in the local shoemaking factories, which accounted for almost half of population, were not admitted to hospitals. Some of the non-natives with dengue returned to their own countries during the early stage of the outbreak, which resulted in loss of data. Second, despite extraordinary efforts to obtain serum specimens, approximately 25% of the residents initially evaluated for dengue refused to provide a convalescent specimen for case definition. Residents in the control area refused to provide speci-

TABLE 3

Survey of serum IgG to dengue virus in the epidemic-stricken and control areas

Survey area	No. positive (men, women)	No. negative (men, women)	Total involved (men, women)	Positive rate (%) (men, women)
Xiaolin	35 (7, 28)	485 (167, 318)	520 (174, 346)	6.7 (4.0, 8.1)
Control	1 (1, 0)	186 (75, 111)	187 (76, 111)	0.5 (1.3, 0)
Subtotal	36 (8, 28)	671 (242, 429)	707 (250, 457)	5.1 (3.2, 6.1)

mens for the serologic survey. Third, because of misdiagnosis at the early stage of the outbreak, we lost some opportunities to sample the sera from patients in the acute phase of the disease for virus isolation.

The dengue outbreak in Ningbo is another example of how readily pathogens can be transferred long distances by travelers to cause outbreaks in new areas.[3,7,16,18,25] What we learned from this episode includes the need to accurately diagnose and rapidly respond to the developments of communicable disease in the global community, the need to maintain surveillance and control potential disease vectors, and the need to provide sanitation education to the public.

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Note: A supplementary table, Dengue serotype-1 sequences retrieved from GenBank, appears online at www.ajtmh.org.

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Authors' addresses: Guozhang Xu, Hongjun Dong, Aiming Zhou, Zhihua Cheng, Jianyi Liu, and Ting Fang, Department of Infectious Diseases, Center for Disease Prevention and Control of Ningbo City, 237 Yongfeng Road, Ningbo, Zhejiang Province, People's Republic of China, E-mails: xugz@nbcdc.org.cn, donghj@nbcdc.org.cn, zhouam@nbcdc.org.cn, chengzh@nbcdc.org.cn, liujy@nbcdc.org.cn, and fangt@nbcdc.org.cn. Nanfeng Shi, Guohua Chen and Jianjie Ye, Department of Infectious Diseases, Center for Disease Prevention and Control of Cixi County, 1141 East Nanerhuan Road, Cixi, Ningbo, Zhejiang Province, People's Republic of China, E-mails: cxcdc@cxcdc.org.cn, hutian11@hotmail.com, and yjj852@sohu.com. Shijian Liu, Hongwei Zhang, Chunying Gu, Xiaojie Tan, and Guangwen Cao, Department of Epidemiology, Second Military Medical University, 800 Xiangyin Road, Shanghai 200433, People's Republic of China, E-mails: arrow64@sohu.com, smmuhongwei@yahoo.com.cn, cygu@smmu.edu.cn, xjtan2003@yahoo.com.cn and gcao@smmu.edu.cn. Shuyun Xie, Department of Infectious Disease, Center for Disease Prevention and Control of Zhejiang Province, 17 Laozheda Road, Hangzhou, Zhejiang Province, People's Republic of China, E-mail: cfetpxsy@vip.sina.com.

Reprint requests: Guangwen Cao, Department of Epidemiology, Second Military Medical University, 800 Xiang-yin Road, Shanghai 200433, People's Republic of China, Telephone: 86-21-25070420, E-mail: gcao@smmu.edu.cn.

REFERENCES

1. Clarke T, 2002. Dengue virus: break-bone fever. *Nature 416:* 672–674.
2. Gubler DJ, 2002. The global emergence/resurgence of arboviral diseases as public health problems. *Arch Med Res 33:* 330–342.
3. Wilder-Smith A, Schwartz E, 2005. Dengue in travelers. *N Engl J Med 353:* 924–932.
4. Qiu FX, Gubler DJ, Liu JC, Chen QQ, 1993. Dengue in China: a clinical review. *Bull World Health Organ 71:* 349–359.
5. Li FS, Yang FR, Song JC, Gao H, Tang JQ, Zou CH, Hu BN, Wen SR, Qiu FX, 1986. Etiologic and serologic investigations of the 1980 epidemic of dengue fever on Hainan Island, China. *Am J Trop Med Hyg 35:* 1051–1054.
6. Qiu FX, Chen QQ, Ho QY, Chen WZ, Zhao ZG, Zhao BW, 1991. The first epidemic of dengue hemorrhagic fever in the People's Republic of China. *Am J Trop Med Hyg 44:* 364–370.
7. Fan WF, Yu SR, Cosgriff TM, 1989. The reemergence of dengue in China. *Rev Infect Dis 11 (Suppl 4):* S847–S853.
8. Luo MH, 2003. *Manual of the Prevention, Control and Treatment for Dengue Fever*. Beijing: China Standard Press, 1–6. (in Chinese)
9. World Health Organization, 1997. Clinical diagnosis. *Dengue Hemorrhage Fever: Diagnosis, Treatment, Prevention and Control*. Second edition. Geneva: World Health Organization, 18–19.
10. Lanciotti RS, Calisher CH, Gubler DJ, Chang GJ, Vorndam AV, 1992. Rapid detection and typing of dengue viruses from clinical samples by using reverse transcriptase-polymerase chain reaction. *J Clin Microbiol 30:* 545–551.
11. Kumar S, Tamura K, Nei M, 2004. MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. *Briefings Bioinform 5:* 150–163.
12. Peng W, Yu M, Fan B, Deng Y, Jiang T, Duan H, Qin E, 2005. Simultaneous infection with dengue 2 and 3 viruses in a Chinese patient returning from Sri Lanka. *J Clin Virol 32:* 194–198.
13. World Health Organization, 1997. Laboratory diagnosis. *Dengue Hemorrhage Fever: Diagnosis, Treatment, Prevention and Control*. Second edition. Geneva: World Health Organization, 43–44.
14. Zhang C, Mammen MP Jr, Chinnawirotpisan P, Klungthong C, Rodpradit P, Monkongdee P, Nimmannitya S, Kalayanarooj S, Holmes EC, 2005. Clade replacements in dengue virus serotypes 1 and 3 are associated with changing serotype prevalence. *J Virol 79:* 15123–15130.
15. Rosen L, Roseboom LE, Gubler DJ, Lien JC, Chaniotis BN, 1985. Comparative susceptibility of mosquito species and strains to oral and parenteral infection with dengue and Japanese encephalitis viruses. *Am J Trop Med Hyg 34:* 603–615.
16. Effler PV, Pang L, Kitsutani P, Vorndam V, Nakata M, Ayers T, Elm J, Tom T, Reiter P, Rigau-Perez JG, Hayes JM, Mills K, Napier M, Clark GG, Gubler DJ, Hawaii Dengue Outbreak Investigation Team, 2005. Dengue fever, Hawaii, 2001–2002. *Emerg Infect Dis 11:* 742–749.
17. Thu HM, Lowry K, Myint TT, Shwe TN, Han AM, Khin KK, Thant KZ, Thein S, Aaskov J, 2004. Myanmar dengue outbreak associated with displacement of serotypes 2, 3, and 4 by dengue 1. *Emerg Infect Dis 10:* 593–597.
18. Nukui Y, Tajima S, Kotaki A, Ito M, Takasaki T, Koike K, Kurane I, 2006. Novel dengue virus type 1 from travelers to Yap State, Micronesia. *Emerg Infect Dis 12:* 343–346.
19. Perret C, Abarca K, Ovalle J, Ferrer P, Godoy P, Olea A, Aguilera X, Ferres M, 2003. Dengue-1 virus isolation during first dengue fever outbreak on easter island, Chile. *Emerg Infect Dis 9:* 1465–1467.
20. Hwang KP, Chu PY, Tung YC, Wang HL, Yueh YY, Wu YC, Chin C, Lin KH, 2003. Molecular epidemiological study of dengue virus type 1 in Taiwan. *J Med Virol 70:* 404–409.
21. Mendez F, Barreto M, Arias JF, Rengifo G, Munoz J, Burbano ME, Parra B, 2006. Human and mosquito infections by dengue viruses during and after epidemics in a dengue-endemic region of Colombia. *Am J Trop Med Hyg 74:* 678–683.
22. Gratz NG, 2004. Critical review of the vector status of *Aedes albopictus. Med Vet Entomol 18:* 215–227.
23. Monath TP, 1994. Dengue: the risk to developed and developing countries. *Proc Natl Acad Sci U S A 91:* 2395–2400.
24. Centers for Disease Control and Prevention (CDC), 2001. Underdiagnosis of dengue – Laredo, Texas, 1999. *MMWR Morb Mortal Wkly Rep 50:* 57–59.
25. Ksiazek TG, Erdman D, Goldsmith CS, Zaki SR, Peret T, Emery S, Tong S, Urbani C, Comer JA, Lim W, Rollin PE, Dowell SF, Ling AE, Humphrey CD, Shieh WJ, Guarner J, Paddock CD, Rota P, Fields B, DeRisi J, Yang JY, Cox N, Hughes JM, LeDuc JW, Bellini WJ, Anderson LJ, SARS Working Group, 2003. A novel coronavirus associated with severe acute respiratory syndrome. *N Engl J Med 348:* 1953–1966.