

Letter to the Editor



Outbreak of Dengue Fever in Central China, 2013*

HUANG Xue Yong^{1,&}, MA Hong Xia^{1,&}, WANG Hai Feng^{1,&}, DU Yan Hua¹,
SU Jia¹, LI Xing Le¹, TANG Xiao Yan¹, MA Hong Peng², ZU Bing Can³,
ZHANG Qiao Hong³, CHEN Hao Min¹, and XU Bian Li^{1,#}

In 2013, the first dengue fever (DF) outbreak in central China was reported in the central of Henan province, northern temperate regions, although they have been sequentially recorded in Southern China. 106 suspected DF cases were reported and 73 patients were confirmed dengue virus type 3 (DEN-3) infections. 62/392 (15.8%) local health persons showed DEN antibodies positive. To this day Henan is the northernmost province in China which has been reported about outbreak of DF and what is important is that it warns us the endemic range of DF has been expanded geographically in China.

Dengue virus (DEN) has a single-stranded positive-sense RNA genome of approximately 11 kb and comprises four distinct serotypes (DEN-1, DEN-2, DEN-3, and DEN-4) which belong to the genus *Flavivirus*, family *Flaviviridae*^[1]. It typically occurs in tropical and subtropical areas in the world and is transmitted by *Aedes* mosquitoes^[1]. Infection with DEN may cause an acute influenza-like febrile disease called classic DF, or the potentially fatal dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). DF is the most rapidly spreading mosquito-borne viral disease in the world^[2-3]. In recent decades dengue outbreaks had been recorded in Guangdong, Hainan, Guangxi, Fujian, and Zhejiang provinces in mainland China^[4-6]. Here, we report details of the first DF outbreak in the central of Henan province, China, northern temperate regions, in late autumn, 2013.

On September 4, 2013, the Yuzhou center for diseases control and prevention (CDC) received a call from a physician in Shenhou township, who had seen about dozens of patients with febrile illness of

unknown origin in the past month. Predominant clinical features of these patients were fever, headache, vomiting, diarrhea, and joint pain. The physician indicated that several of the patient's family members had become symptomatic. DEN was not the first etiological hypotheses because outbreak of DF had not been reported in Henan though there were cases of imported dengue infection reported sporadically. Shenhou township located in Yuzhou city (33-34 degrees north latitude), in the central of Henan province, China. Exclusion of several prevalent febrile illnesses locally, combining with the clinical characteristics, on investigation by Henan CDC staff, dengue fever was suspected and clinical specimens were collected and transported to Henan CDC for diagnosis.

All acute serum samples from suspected DF case, including healthy persons who had been present with DF like illness history in the last two months, were screened for DEN RNA (DEN-1,2,3,4) by real-time RT-PCR (Shuoshi) and dengue virus recovery by inoculating the susceptible cell line Vero. The DEN immunoglobulin (Ig)M capture ELISA (Panbio) and indirect IgG ELISA (Panbio) were used for qualitative detection of anti-DEN IgM and IgG antibody in serum, as an aid in the laboratory diagnosis of patients with clinical symptoms consistent with DEN infection. And the DEN capture IgG ELISA (Panbio) were used for detection of secondary DEN infection. We defined a laboratory-confirmed case who presenting with clinical symptoms consistent with DEN infection as meeting one or more of the following criteria: the isolation of DEN from the patient's serum, the

doi: 10.3967/bes2014.125

*This work was sponsored by Henan Province Health Department and China's Ministry of Health Co-build Project (201201003) and Henan Department of Science and Technology Project (122400450357). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

1. Henan Center for Disease Control and Prevention, Zhengzhou 450016, Henan, China; 2. Yuzhou Center for Disease Control and Prevention, Yuzhou 461670, Henan, China; 3. Xuchang Center for Disease Control and Prevention, Xuchang 461000, Henan, China

detection of DEN RNA in the patient's serum, or the detection of IgM or IgG antibodies against DEN in serum. 4 of 12 positive isolates were sequenced (Accession numbers: KJ622191-KJ622194). Nucleotide sequences were analyzed with the MEGA 4.0 software package and DNASTAR 5.0 software.

To assess the extent of DEN transmission and population seroprevalence of dengue, we conducted seroepidemiologic studies among close contacts exposed to DF cases and nearby residents in Shenhou township and 392 serum samples were collected from the health persons which were randomly selected in seven villages in Shenhou township. 302 sera collected from these health residents who lived in the four villages (Miaojiawan, Luowang, Yangling, Zhujiashan) (Figure 1) with reported cases and 56 of them were from patients' household contacts (33 families), that is, these health persons shared residences with patients. There was even more than one case in some families. The other 90 serum samples were collected from three neighboring villages of Miaojiawan and no patient was reported in these villages. All of these sera were tested for anti-DEN IgM and IgG antibody by using DEN IgM capture ELISA (Panbio) and indirect IgG ELISA (Panbio).

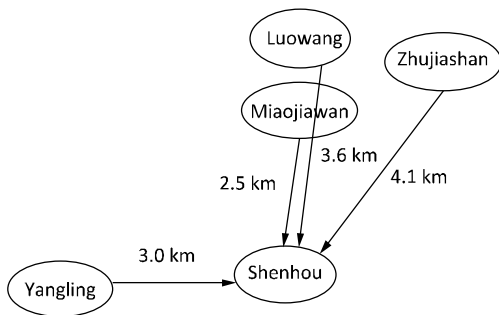


Figure 1. Schematic of Miaojiawan, Luowang, Yangling, and Zhujiashan villages in Shenhou township.

The first DF case with clinical characteristics was tested for anti-DEN antibody and confirmed on September 13, 2013 and then we confirmed that the Shenhou cases started on July 25 by retrospective survey. The last DF case was found on September 18, 2013 and no death was reported in this outbreak. A total of 106 suspected DF cases, including healthy persons who had been present with dengue like illness history in the last two months, were reported in this outbreak in four villages in Shenhou township. There were no samples available for 22 of them and 114 serum samples were collected from 84 of them, including 27 acute-phase serum samples collected from patients within one week after illness onset, 87 sera from suspected cases who had recovered or more than one week after onset and 30 paired sera of them.

12 strains of DEN were isolated from 27 sera which were collected within one week after onset and all of these 27 acute sera showed IgG antibody negative with DEN capture IgG ELISA (Panbio). 4/12 isolates were sequenced and BLAST results showed that they have the highest similarity (98%) to the Thailand strain ThD3_0010_87 (AY676352) isolated in 1987 based on the complete genome. Additionally, epidemiological data showed that several villagers had traveled from Laos to Miaojiawan, the village with the most cases, in late May. 26/84 serum samples and 12 isolates tested positive for DEN-3 RNA. 61/84 showed detectable anti-DEN IgM and/or IgG antibody (Table 1) and 12/84 showed DEN-3 RNA positive without anti-DNE antibody. In all, there were 73/84 patients were identified as laboratory-confirmed DEN infection cases. These suspected DF patients had been reported in four villages and 71/73 laboratory confirmed DF cases lived in one village named as Miaojiawan. The ratio of female to male was 1.55:1 for 56 confirmed DEN patients whose demographic information was available, and most patients were 30 to 70 years of age (73.2%).

Table 1. IgM/G ELISA and Real-time RT-PCR Results for 84 Patients with Suspected Dengue Virus Infection

Characteristic	No. Antibody Positive Patients			No. Antibody Negative Patients	Total
	IgM ⁺ &IgG ⁻	IgM ⁺ &IgG ⁺	IgM ⁻ &IgG ⁺		
No. nucleotide positive patients	6	1	7	12	26
No. nucleotide negative patients	9	2	36	11	58
Total	15	3	43	23	84

62 of 392 (15.8%) (Table 2) healthy peoples showed IgM/G DEN antibodies positive. The highest prevalence of anti-DEN antibodies as 19.6% (11/56) was in these people who lived in the same houses, followed by people who lived in the same villages with DF patients as 17.1% (42/246). A seroprevalence of 10.0% (9/90) was observed in the other three villages without report cases. For all of these 62 asymptomatic DEN infection peoples, 23 peoples were detected but DEN IgM antibody not DEN IgG antibody, 28 peoples were detected both DEN IgM and IgG antibody positive and 11 peoples showed just DEN IgG antibody positive (Table 3). All of these anti-DEN IgM antibody positive sera were anti-JE IgM antibody negative, test by ELISA (Shanghai beixi) and JE case had not been reported in Shenhou since 2000.

This is the first report of DF outbreak in Henan in central China although they have been sequentially recorded in Southern China since 1978^[4-6]. There were a lot of people in Shenhou township to go to southern coastal regions of China, even neighboring country, such as Laos, for work or trade. We

speculate that imported cases had caused the epidemic with local infection outbreaks. DF was uncommon for local physician and its clinical symptom was mostly mild and self-limiting. Consequently, it had not been taken enough notice, then, it got enough time to transmit and be resulted in this outbreak.

To the serological epidemiology investigation in healthy people, there were 52 peoples showed anti-DEN IgM antibody positive, even 23 out of these 52 peoples were detected anti-DEN IgG negative, which was represent recent dengue infection. We know that the positive rate of 15.8% is pretty high for the areas never reported before. We thought of two reasons for this result: One was that it was possible that DEN infection was under or miss-diagnosis in the past in these areas. After all, its clinical symptom was mostly mild and self-limiting. The other one maybe was that the 'random sampling' was limited. we conducted seroepidemiologic studies mainly among close contacts exposed to DF cases, such as 56/392 were family members of patients and 246/392 were patients' villagers.

Table 2. Population Characteristics and Prevalence of IgM/G Dengue Antibodies by Degree of Exposure

Characteristic	No. Positive Samples/No. Tested (%)			Total (%)
	Patients' Household Contacts (%)	Patients' Villagers (%)	Residents in Neighboring Villages (%)	
Age group (y)				
0-10	1/4 (25.0)	2/18 (11.1)	3/11 (27.3)	6/33 (18.2)
11-20	0/3 (0)	0/2 (0)	0/7 (0)	0/12 (0)
21-30	0/8 (0)	0/17 (0)	0/7 (0)	0/32 (0)
31-40	1/9 (11.1)	4/29 (13.8)	0/10 (0)	5/48 (10.4)
41-50	3/9 (33.3)	9/65 (13.8)	2/29 (6.9)	14/103 (13.6)
51-60	0/10 (0)	12/51 (23.5)	3/14 (21.4)	15/75 (20.0)
61-70	4/8 (50.0)	7/33 (21.2)	0/4 (0)	11/45 (24.4)
≥70	2/5 (40.0)	5/20 (25.0)	1/8 (12.5)	8/33 (24.2)
*	0/0 (0)	3/11 (27.3)	0/0 (0)	3/11 (27.3)
Sex				
male	5/29 (17.2)	18/99 (18.2)	2/31 (6.5)	25/159 (15.7)
female	6/27 (22.2)	24/140 (17.1)	7/59 (11.9)	37/226 (16.4)
*	0/0 (0)	0/7 (0)	0/0 (0)	0/7 (0)
Total	11/56 (19.6)	42/246 (17.1)	9/90 (10.0)	62/392 (15.8)

Note. * No available data of age or sex.

Table 3. Serologic Test Results for Serosurvey in Shenhou, 2013

Characteristic	IgM ⁺ & IgG ⁻	IgM ⁻ & IgG ⁺	IgM ⁺ & IgG ⁺	IgM ⁻ & IgG ⁻
Patients' household contacts	3	0	8	45
Patients' villagers	16	7	19	204
Residents in neighboring villages	4	4	1	81
Total	23	11	28	330

Unfortunately, no *Aedes* mosquitoes were captured by us in Shenhou township. But the strong household clustering of DEN infection suggests that family sources of exposure are important. We found that there was more than one DEN infection member in many families and most of them had not even left Shenhou township. As it was clearly, most of them were infected in Shenhou. So we speculate that DEN-carrying mosquitoes were the family sources of exposure. As we know, the only way to prevent the spread of DEN is to fight the mosquitoes that carry them.

Taking both the travel history of local residents and the molecular characteristics of these isolates, it is likely that this outbreak was the result of virus introductions from Southeast Asia or southern coastal regions of China, cases began in Miaojiawan and subsequent infections were transmitted to other villages of Shenhou township. To this day Henan is the northernmost province in China which has been reported about outbreak of DF and what is important is that it warns us the endemic range of DF has been expanded geographically in China.

ACKNOWLEDGMENTS

We sincerely acknowledge the cooperation of Xuchang and Yuzhou Centers for Disease Control and Prevention in this study.

COMPETING INTERESTS

None declared.

ETHICAL APPROVAL

This research was approved by the Review Board of Henan Center for Disease Control and Prevention. Informed consents were already taken from the participant prior to study.

[#]Correspondence should be addressed to XU Bian Li, Tel: 86-371-68089291, Fax: 86-371-68089291, E-mail: bianlixu@163.com/bianlixu@gmail.com

[&]These authors contributed equally to this article.

Biographical notes of the first authors: HUANG Xue Yong, male, 1972, PhD in epidemiology; MA Hong Xia, female, 1981, PhD in molecular biology; WANG Hai Feng, male, 1976, Master of epidemiology.

Received: June 9, 2014;

Accepted: September 1, 2014

REFERENCES

1. Holmes EC, Twiddy SS. The origin, emergence and evolutionary genetics of dengue virus. *Infect Genet Evol*, 2003; 3, 19-28.
2. Ferreira GLC. Global dengue epidemiology trends. *Rev Inst Trop Sao Paulo*, 2012; 54, 5-6.
3. Murray NE, Quam MB, Wilder-Smith A. Epidemiology of dengue: past, present and future prospects. *Clin Epidemiol*, 2013; 5, 299-309.
4. Wu JY, Lun ZR, James AA, et al. Dengue fever in mainland China. *Am J Trop Med Hyg*, 2010; 83, 664-71.
5. Peng HJ, Lai HB, Zhang QL, et al. A local outbreak of dengue caused by an imported case in Dongguan China. *BMC Public Health*, 2012; 12, 83.
6. Zhang H, Zhang Y, Hamoudi R, et al. Spatiotemporal Characterizations of Dengue Virus in Mainland China: Insights into the Whole Genome from 1978 to 2011. *PLOS one*, 2014; 9, e87630.