Short Communication

The First Measles Outbreak Caused by Imported Genotype D9 Measles Virus in Shandong Province, China, 2013

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SUMMARY: This study represents a measles outbreak caused by the genotype D9 measles virus (MeV), which was imported by Burmese individuals. Urine and throat swab specimens were collected from suspected measles cases. Viruses were isolated, and a 634-bp target fragment of the N gene was amplified by reverse transcription-PCR and sequenced. Phylogenetic results indicated that the 6 isolates belonged to genotype D9 MeV. Through appropriate prevention and control measures, the transmission of genotype D9 MeV was interrupted. Genotype D9 MeV was isolated for the first time in Shandong and was imported by Burmese individuals.

Measles virus (MeV) is a negative-strand RNA paramyxovirus, which causes an acute infectious disease and a chronic clinical disorder. More than 30 million new cases are reported annually worldwide, with the majority being children (1,2). In 2010, China implemented a nationwide measles mass immunization campaign (MMIC) designed to administer a dose of catch-up measles vaccine to all age-eligible children, free of charge. Coinciding with the development of the MMIC program, the measles morbidity and mortality have substantially declined since 2010 (3,4).

MeV has a single serotype; however, it has 24 genotypes circulating globally. MeV surveillance in China was initiated in 1993 and demonstrated that genotype H1 MeV was predominantly circulating in China (5). However, several MeV genotypes, including D4, D9, and D11, were isolated from local Chinese residents during 2009–2012 (6–9). One sporadic measles case associated with genotype D9 MeV was reported in Sichuan Province in 2009; this was the first genotype D9 MeV detected in China and was confirmed to have been imported from Thailand (7). In 2012, a case with genotype D9 MeV was identified in Yunnan Province bordering Myanmar (8). Here we describe, for the first time, the isolation of genotype D9 MeV from 4 adults who traveled 2,250 miles to Yantai, Shandong, China from Kokang, Myanmar in March 2013. Importantly, the same genotype D9 MeV was isolated from their contacts and the transmission of genotype D9 MeV was interrupted.

The study (including the written informed consent and investigation questionnaire) was approved by the Shandong Center for Disease Control and Prevention (CDC) Ethics Committee for Human Research on Preventive Medicine. Written informed consent was obtained from all the patients in this study for the collection of specimens and the investigation.

Specimens were collected from 6 patients. Epidemiological investigation was performed by the Zhifu CDC. Throat swab and urine specimens were obtained from the patients within 5 days of rash onset. The Vero/SLAM cell line (African green monkey kidney cells transfected with a plasmid encoding the gene for the human signaling lymphocyte activation molecule) was used for the isolation of MeV, and the infected cells were harvested when the cytopathic effect (CPE) was visible over 75% of the cell layer.

The QIAamp® Viral RNA Mini Kit (Qiagen, Hilden, Germany) was used to extract MeV according to the manufacturer’s instructions. Reverse transcription (RT)-PCR amplification was performed using previously described primers (10) to amplify a 634-bp fragment of the N gene, which included a 450-bp fragment recommended for genotyping.

The PCR products were sequenced by automated sequencing and the BigDye terminator v3.0 chemistry according to the manufacturer’s protocol in both sense and antisense strands using an automated ABI PRISM® 3100 DNA Sequencer (Applied Biosystems, Foster City, CA, USA). The sequence proofreading and editing was conducted with Sequencher 4.0 software (Gene Code, Ann Arbor, MI, USA). Subsequently, multiple sequence alignment was performed and phylogenetic tree was constructed using MEGA software, version 4.0.
Imported Measles Virus Genotype D9

Epidemiological investigation indicated that 4 index cases were from Kokang, Myanmar; they developed fever and rash during March 1–2. The recruiters of the enterprise in Yantai recruited more than 50 workers in Yunnan, including the 4 Burmese. They gathered from their hometown to Kunming, Yunnan on March 1 and took a chartered bus and reached Yantai on March 3. The other 2 local cases were Yantai residents, who developed fever and rash on March 20 and April 2, respectively. One of the local resident cases was a male worker
who worked in the same enterprise as the 4 index cases, and the other was a local health worker who worked at a dental clinic in Yantai. Measles vaccination history was unknown in all the 6 patients. Five of the 6 patients presented with typical clinical signs of measles, including Koplik’s spots.

Both IgM test and real-time RT-PCR detection confirmed that all the 6 patients had measles. The 634-bp target fragment could be amplified in 6 throat swab specimens and 3 out of 4 urine specimens from the 6 patients. According to the standard naming method for wild MeV strains recommended by the World Health Organization (WHO), the 6 MeV strains were named as MVi/Yantai.SD.CHN/12.13/9 (KF929545), MVi/Yantai.SD.CHN/12.13/10 (KF929546), MVi/Yantai.SD.CHN/12.13/11 (KF929547), MVi/Yantai.SD.CHN/12.13/12 (KF929548), MVi/Yantai.SD.CHN/14.13/35 (KF929549), and MVi/Yantai.SD.CHN/14.13/36 (KF929550). At the same time, a predominant strain of MeV in Shandong Province was obtained and named as MVi/Yantai.SD.CHN/14.13/37 (KF929551).

The coding regions of 450 nucleotides at the COOH terminal of the N protein are the minimum amount of sequence data required for assigning a measles genotype (11). We blasted the 450 nucleotides encoding the COOH terminal of the MeV N protein and constructed thephylogenetic tree (Fig. 1) consisting of the 6 isolates and 24 WHO reference strains using MEGA software, version 4.0. After the outbreak, the Shandong CDC took rapid measures to interrupt the transmission of genotype D9 MeV, and subsequently, this genotype could not be detected in Shandong Province.

Shandong Province participated in the nationwide synchronized measles supplemental immunization activities (SIAs) in 2010. The vaccination coverage rate was more than 95%. Following SIAs, the reported measles cases were 483 in Shandong in 2011, which is 72.7% decrease compared with the number of cases in 2010. In 2012, the reported incidence of measles in Shandong Province was 2/10,000,000, which represented 21 cases. Shandong Province is the first province in China to have initiated MeV surveillance since 1993. The continuous surveillance lasting for 2 decades revealed that genotype H1 is predominant in Shandong.

Yantai is a developed coastal city of Shandong Province, where routine immunization and measles surveillance have been well implemented. Estimated vaccination rates are more than 95%, and the incidence of measles has been pretty low in recent years. The reported incidence of measles in Yantai in 2011 and 2012 was 1.1/1,000,000 and 1.4/10,000,000, respectively.

The endemic genotype H1 MeV has been circulating in mainland China for at least 16 years (3,12). Other genotypes, including D9 MeV, were associated with Chinese residents who either traveled internationally or lived near a bordering country from where the virus was imported. A case with genotype D9 MeV was identified and confirmed to be sporadic in January 2012 in Yunnan; however, following this, no cases of this genotype were identified in Yunnan until May 2013 according to Yunnan provincial measles surveillance data. In the present study, 4 of the patients were recruited into an enterprise of Yantai from a bordering town of Yunnan, and epidemiological investigation confirmed that these patients were Burmese and contracted MeV infection in Myanmar before March 1 and arrived in Yantai on March 3 with clinical symptom of measles. Thus, we believe that this measles outbreak that occurred in Yantai was associated with genotype D9 MeV that originated from 4 Burmese individuals who were infected in Myanmar, and that importation caused the spread of genotype D9 MeV.

Based on the routine WHO measles surveillance guideline, MeV was isolated first and then genotyped on the basis of the 3’-end partial N gene. Usually, it takes 2 months to report the laboratory findings. In the present study, we adopted the revised virus surveillance strategy suggested by the National Measles Reference Laboratory of the China CDC located in Beijing. In brief, fluorescence-based real-time RT-PCR was used for the detection of the MeV gene. The positive specimens were then subjected to RT-PCR to directly amplify the sequencing target fragment. The revised test procedure improves the sensitivity and timeliness of MeV detection, thereby facilitating public health response and outbreak control (13).

Nucleotide and amino acid homology analysis revealed that the 450-nucleotide sequence was identical among the Yantai genotype D9 MeV isolated in the present study, Yunnan D9 isolated in 2012, and genotype D9 found in Thailand, Netherlands, and Russia in recent years, indicating the universality and stability of genotype D9 MeV during transmission. However, the Yantai genotype D9 MeV differed from those isolated in Sichuan Province of China in 2009, Ningxia autonomous region in 2010, Hong Kong in 2012, and Malaysia in 2011 owing to different sources of MeV or variation during transmission. The global MeV surveillance network has collected MeV genotype data in recent years and determined the distribution characteristics of MeV genotypes around the world. MeV surveillance in different countries has proven to be extremely useful for tracking global transmission pathways or documenting the interruption of MeV transmission (14).

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Conflict of interest None to declare.

REFERENCES