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Molecular Characterization of Wild Type Measles Virus from Adult Patients in Northern China, 2014

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ABSTRACT

Objectives: In this study, we studied the N and H genes from wild type measles viruses (MeVs) isolated during the 2013-2014 outbreak.
Methods: Clinical samples were collected, and the genotyping, phylogenetic analysis were performed.
Results: The vaccination rate of the study population was 4%. Genotype H1a was the predominant genotype. Wild type viruses were classified into clusters A and B, C and may have different origins. N-450 sequences from wild type viruses were highly homologous with, and likely evolved from MeVs circulating in Tianjing and Henan in 2012. MVs/Shenyang.CHN/18.14/3 could have evolved from MeVs from Liaoning, Beijing, Hebei, Heilongjiang, Henan, Jilin, and Tianjin. Our data suggested that one or more of the same viruses circulated between Beijing, Shenyang, Hong Kong, Taiwan and Berlin.
Conclusions: Important factors contributing to outbreaks could include weak vaccination coverage, poor vaccination strategies, and migration of adult workers between cities, countries, and from rural areas to urban areas.

1. Introduction

Measles is the leading cause of vaccine-preventable childhood morbidity and mortality globally,[1] and remains a major public health concern in China because of frequent outbreaks. The measles virus (MeV), a member of the genus Morbillivirus of the family Paramyxoviridae is an enveloped virus with a non-segmented negative-sense RNA genome.[2] MeV is highly contagious and causes a disease characterized by high fever, cough, coryza, conjunctivitis and appearance of a maculopapular rash.

MeV is a monotypic virus, but recent reports have described 24 different genotypes in the 450 nucleotide region coding for 150 amino acids of the carboxy terminus of the nucleoprotein (N-450) and 1854 nucleotides encoding the entire hemagglutinin protein (H).[3] Antigenic variability is thought to contribute to the spread of MeV in vaccinated populations. Indeed, MeVs which were variants in the N and H genes were isolated during the outbreak in Jilin Province during 2005-2006, where the post-vaccination sera had diminished neutralizing titers against wild-type strains.[4] Molecular epidemiological studies of MeVs have therefore emerged as an important means of establishing links, tracing transmission pathways, allowing detection of imported cases, and classification of suspected cases as caused by vaccine or wild type strains.[5] Virologic surveillance has shown that genotype H1 MeV has been the predominant strain circulating in China since 1993, and is endemic throughout China.[6] Based on phylogenetics analyses, genotype H1 MeVs are classified as 1) Cluster1 viruses (H1a), which comprise the most frequently detected strains since 2000, and 2) Cluster2 viruses (H1b), which have not been detected after 2005.[7–10]

Previous reports suggested that immunization of populations with low vaccination coverage is vital since these populations represent a bigger public health risk than sporadic, susceptible individuals.[11] China initially implemented a two-dose measles vaccination program in 1986, where the first dose was administered to infants at 8 months of age, and the second dose at 7 years of age.[12] The current vaccination strategy consists of two doses of the measles containing vaccine (MCV1 and MCV2) administered at 8 months and 18-24 months of age. Non-vaccinated adults and

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subjects whose immunization history is unknown receive one dose of the measles-rubella combination vaccine.[13] The Chinese government has expanded routine immunization programs and has conducted large-scale supplementary immunization activities (SIAs) in order to meet the regional measles elimination goals set by the WHO Regional Committee of the Western Pacific Region (WPR) in 2005. In 2006, China endorsed the 2006-2012 national action plan for elimination of measles.[14] Based on the measles incidence and the presence of susceptible populations, province-specific as well as nationwide SIAs have been conducted between 2004-2010, where > 280 million children and adolescents were vaccinated. Although the goal of the vaccination program was to achieve a coverage of >95%, a number of epidemiological studies showed that this was not achieved. The rates of estimated coverage for MCV1 and MCV2 in 2009 were reported to be 91.1% and 84.3%, respectively,[13] with more than 52,000 cases being reported in 2009 alone. Another recent study showed that the rates of coverage for MCV1 and MCV2 were 99.4% and 93.35%, respectively.[15] A study which estimated vaccination coverage in the 2010-2012 birth cohort showed that the estimated coverage rates for MCV1and MCV2 were 84.1% - 87% and 80.3%-90%, respectively.[16] Barriers such as the mother's education level, household income, multiple children in the household, and the child being sick during the vaccination period have been cited as significant barriers to timely vaccination among migrant populations. The MCV1 and MCV2 coverage rates among migrant children were only 76.9% and 4.7%, respectively.[17]

In China, a measles outbreak is defined as the occurrence, within a 10-day period, of either at least two confirmed cases in a village, district, school or similar unit, or at least five confirmed cases in a township. Outbreaks have been attributed to a number of reasons including 1) failure of the primary vaccine, 2) an unvaccinated population living in remote regions and 3) human migration.[4] Failure of vaccination can also be due to factors such as age of first vaccination, the number of doses administered to children (1 or 2 doses) and a cold chain of vaccine.

Although the incidence of measles in China decreased dramatically from 99.4 per million in 2008 to 4.6 per million in 2012, there have been frequent reports of indigenous measles virus outbreaks. There have been a total of 914 measles outbreaks with an onset in 2005 throughout the 31 provinces in China. Of these cases, 37%-50% were estimated to have occurred in migrant populations.[12] A total of 26,000 cases were reported between January and October 2013 of which 68% of the patients were < 5 years old.[14] Measles outbreaks were reported in Beijing and Shenyang in late 2013 and 2014, where about 91.8% of all patients were adults (age >18 years old, unpublished data). The Beijing Public Health Information Center recorded more than 1070 measles cases between January to April, 2014 in Beijing. The recent increase in the number of outbreaks has been attributed to a number of reasons including vaccination coverage, and gap in population immunity, and suggests that there is an urgent need to evaluate and address challenges in identifying immunity gaps in the general population.

In this study, we investigated the molecular characteristics of the MeV N and H genes from wild type viruses isolated during the 2013-2014 outbreak, in order to understand the source of the virus, trace possible transmission pathways, and to monitor the variations of neutralizing targets. We also investigated whether variation in genotype could reduce vaccine efficacy.

2. Materials and methods

2.1. Case selection and sample collection

This study enrolled a total of 327 adult inpatients and outpatients (age > 18 years old) who presented with measles at the Beijing 302 Hospital and the Shenyang Sixth People's Hospital between December, 2013 and September 2014. A clinical diagnosis of measles was based on the presence of fever, maculopapular rash and at least one of the following symptoms: cough, coryza or conjunctivitis. The diagnosis was confirmed by the presence of MeV IgM antibodies in serum. Throat swabs for virus isolation and RNA extraction were collected from all patients at their first visit to the doctor or at the inpatient departments. The samples were stored at −80 °C.

2.2. Detection of measles virus IgM antibodies

Serum samples were tested for IgM antibodies specific for measles virus using the Enzygnost Anti-Measles Virus/IgM kit according to the manufacturer's instructions (Siemens Health Care Diagnostics Products, GmbH).

2.3. RT-PCR amplification and sequencing

Total RNA was extracted from throat swab samples using the QIAmp Viral RNA Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. The 594 bp fragment from the 3′ region of the N gene which contains the N-450 region was amplified using the forward primer (N-450U: 5′-GCTATGCCATGG-GAGTAGGAGTGG-3′) and reverse primer (N-450L: 5′-CCTCGGCCTCTCGCACCTAGT-3′). The 1914 bp fragments including entire H gene 1854 bp was amplified using forward primer (5′-CATCCACAA TGTCACCACAA-3′) and reverse primer (5′-GTGGGTATGCCTGATGTCTG-3′). All RT-PCR reactions were performed on 0.5 μg total RNA for reverse transcription using Easy Script reverse transcriptase ((Transgen, Beijing, China) _and followed by PCR using 1 μg products of transcription and the TransStart fast Pfu DNA Polymerase (Transgen, Beijing, China) according to the manufacturer's instructions. The 594 bp of the N gene was sequenced with the N450U primer, and the entire H gene was sequenced using the forward and reverse primers as well as 4 sequencing primers (Hs1: 5′-GTCAGAGATGAATTTCAC-3′, Hs2: 5′-TTGGTGAACTCAACTCTACTG-3′, Hs3: 5′-GGA ACTGAGTTTGA-CATCAC-3′, Hs4: 5′-GTATGCCTGATGTCTGGGTGA-3′). Of these 262 samples, RT-PCR were amplified successfully in 82 cases for N-450 sequencing, and in 42 cases for sequencing and evaluating H gene variations. Failure of RT-PCR in the remaining samples could have been either due to poor sample quality, or low copy number of the virus.

2.4. Sequence and phylogenetic analysis

Sequence data were analyzed using Clustal W program implemented in MEGA (version 6) for multiple alignments. Phylogenetic analysis used MEGA. Dendrograms were drawn using the neighbor-joining method (1000 bootstraps). The new MeV strains obtained from the outbreaks in Beijing and Shenyang were named as designated by the WHO. The N-450 sequences from 21 representative wild type MeV strains accessed from Genbank as well as from 24 WHO reference genotype strains and 1 Chinese vaccine strain (S191) were used to construct the phylogenetic trees. The sequence alignment of H proteins was processed by Bioedit (version 7).

3. Results

3.1. Patient demographics and serology

This study enrolled 327 adult patients with measles. The study population comprised 167 males and 160 females (Table 1). Most of the patients were 18-58 years old and the median age was 34