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Measles incidence rate and a phylogenetic study of contemporary genotype H1 measles strains in China: is an improved measles vaccine needed?

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Abstract The incidence of measles in China has increased over the last decade. To evaluate the genetic variation of measles strains, 16 measles wild-type virus strains were isolated from 14 vaccinated cases and 2 nonvaccinated cases in Jilin Province during 2005–2006, and their nucleoprotein (N) and hemagglutinin (H) genes were amplified by RT-PCR. The amplified products were sequenced and compared with the Edmonston virus and the existing vaccine strains (Changchun-47 and Shanghai-191). The results showed that the variation rate between the vaccine and wild-type strains was 9.8–12.0% in the N gene and 5.9–6.9% in the H gene, respectively. In addition, cross-neutralization assays revealed that although sera obtained from infants following primary vaccination effectively neutralized vaccine strains, the capacity in neutralizing H1 wild-type measles virus isolates was decreased fourfold. Antigenic ratios testing revealed that the antigenic relatedness between wild-type measles viruses and existing vaccine strains was notably low. These data suggest that the increased incidence of measles in Jilin Province may be attributed to the antigenic drift between wild-type and vaccine strains. Our findings strengthen the recommendation of supplemental immunization with existing vaccines and also strongly suggest a need for developing new vaccines to better control measles virus outbreaks.

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Keywords Measles virus · Nucleotide variation · Vaccine protection

Abbreviations

MV	Measles virus
SLAM	Signaling lymphocyte-activation molecule
RT-PCR	Reverse transcription-polymerase chain reaction
CPE	Cytopathic effect
R	The antigen ratio
Edm	Edmonston
MEGA	Molecular evolutionary genetics analysis
GMT	Geometric mean titers

Introduction

Measles virus (MV) belongs to Morbillivirus genus in the family of Paramyxoviruses. Although genetic heterogeneity exists in natural MV strains, MV is considered a single-serotype [1]. The clinical manifestations of measles include fever, coryza, cough, and conjunctivitis followed by the appearance of a generalized maculopapular rash. MV infects approximately 30 million people with mortality of 197,000 annually, which occurs mainly in developing countries [2]. In the pre-vaccine era, more than 90% of 15-year-old children had a history of measles [3], and measles was a major cause of mortality in children. Measles incidence was greatly decreased after measles vaccines were introduced. In China for example, measles morbidity and mortality have dramatically decreased since the introduction of measles vaccine in 1965, and further reduced after the implementation of routine infant vaccination in 1978 and the use of a two-dose schedule

(administered at 8 and 18–24 months of age) since 1990. It has been estimated that measles vaccination coverage for infants is currently above 98.5% in China. The World Health Organization (WHO) developed a plan to eliminate measles worldwide by 2012. However, China is still facing challenges of tackling measles virus because over 70,000 measles cases and 26 measles related deaths were reported in 2004 and more than 52,000 measles cases in 2009 [4]. The complication of measles control in China includes primary vaccine failure and a largely unvaccinated population, such as those living in remote rural regions, urban homeless, and migrants. Nevertheless measles outbreaks still occur even under high vaccination coverage in China [5], suggesting that current measles vaccines may not be effective against circulating strains. It remains unknown that whether antigenic variation in contemporary measles virus strains plays a role in allowing measles virus to spread out within highly vaccinated populations.

There are 23 genotypes of measles virus, all of which are recognized by WHO [6], and five of them (B1, D1, E, F, and G1) are considered inactive since they have not been detected for the past 15 years [7]. In China, a recent analysis of circulating measles virus strains led to the identification of a new clade, "hemagglutinin (H)" [8, 9]. The genotype H1 has been considered as the only endemic genotype widely distributed across China. Interestingly, China's neighboring countries have different epidemic genotypes: D4 and D8 in Nepal, D4 in Pakistan, G2 in Thailand, and H2 in Vietnam.

In this study, we investigated possible causes which may have contributed to increased incidence rate of the measles in Jilin Province, China during 2005–2006. We isolated 16 measles virus isolates from 105 patient samples and sequenced them. Our results showed that nucleotide variation occurred in both nucleoprotein (N) and H genes with rates of 9.8–10.5% and 11.3–12.0% in N gene and 6.2–6.9% and 5.9–6.7% in H gene compared with two existing genotype A vaccine MV strains (Changchun-47 and Shanghai-191). We also found that the ability of post-vaccination sera to neutralize new H1 MV isolates was significantly decreased following primary vaccination when compared with the ability to neutralize the homologous vaccine strain. Our findings suggest that wild-type MV gene variation and antigenic drift between wild-type and vaccine MV strains may contribute to the increase in the numbers of measles cases reported in China recently. Thus, there may be a real need not only for enhancing supplemental immunization programs but also for the development of new measles vaccines to address the global health problem.

Materials and methods

Cell culture

Vero/SLAM (signaling lymphocyte-activation molecule) cell line was used to isolate MV from clinical specimens [10]. Vero/SLAM cells were cultured in Dulbecco's modified minimal essential medium (DMEM, GIBCO-BRL) supplemented with 10% fetal calf serum (FCS, GIBCO-BRL), 100 U/ml penicillin and 100 μg/ml streptomycin and 5% CO_2 at 37°C. To maintain selection pressure, 1 mg/ml G418 was added to the growth medium. These cells steadily express MV N and phosphoprotein (P) as well as T7 RNA polymerase.

Sample collection, virus isolation, and epidemiology

This study was approved by the Ethics Committee of Jilin University and informed consent was obtained from all participants. Clinical samples (throat swab and urine) were collected from patients with typical measles symptoms, including high fever, cough, conjunctivitis, Koplik's spots on the buccal mucosa, and rash, and were transported according to standard protocols. Then the collected specimens were inoculated onto Vero/SLAM cells prepared as previously described [7]. The infected cells were harvested when cytopathic effect (CPE) reached the maximal level, while if no CPE was observed, blind-massaged up to three times before discarding [11].

Serum samples from measles cases were collected as part of the clinical investigation for diagnosis. Moreover, sera were also collected from healthy infants with pre- or post-vaccination and healthy adults aged 20–40 years after parents/guardians or adult subjects provided informed consent for serological testing.

RNA extraction and RT-PCR

MV-RNA was extracted from infected cell suspension using MiniBEST Viral RNA/DNA Extraction Kit Ver.4.0 according to the manufacturer's instructions (TaKaRa). Following extraction, the H and N genes were amplified by RT-PCR using the TaKaRa One Step RNA PCR Kit (AMV). The Primer-F_N (5′ GCT ATG CCA TGG GAG TAG GAG TGG 3′), Primer-R_N (5′ GGC CTC TCG CAC CTA GTC TAG 3′), Primer-F_H (5′ AAA CTT AGG GTG CAA GAT CAT CC 3′), and Primer-R_H (5′ ACA TCA TGA GAT TGG TTC ACT AGC 3′) [12] were derived using consensus sequences obtained from MV genotypes H1 and A (Edmonston) strains.

Table 1 Summary of natural measles virus strains isolated in Jilin Province, China in 2005–2006

No. in Lab	Standard name of measles virus	City	Epidemic character	Sample	Age	Vaccination[a]	Genotype
Jlu-1	Mvi/Changchun.PRC/07.05/15	Changchun	Sporadic	Throat swab	27 m	1 dose	H1
Jlu-2	Mvi/JiIinshi.PRC/09.05/15	Jilin	Outbreak	Throat swab	24 m	1 dose	H1
Jlu-3	Mvi/Jilinshi.PRC/11.05/14	Jilin	Outbreak	Throat swab	21 y	2 doses	H1
Jlu-4	Mvi/Yanbian.PRC/12.05/16	Yanbian	Sporadic	Throat swab	7 m	Nonvaccinated	H1
Jlu-5	Mvi/Tonghua.PRC/15.05/23	Tonghua	Outbreak	Urine	16 y	1 dose	H1
Jlu-6	Mvi/Siping.PRC/16.05/6	Siping	Outbreak	Throat swab	24 m	1 dose	H1
Jlu-7	Mvi/Siping.PRC/17.05/33	Siping	Outbreak	Urine	37 m	1 dose	H1
Jlu-8	Mvi/Siping.PRC/21.05/2	Siping	Outbreak	Urine	21 m	1 dose	H1
Jlu-9	Mvi/Changchun.PRC/21.05/19	Changchun	Outbreak	Throat swab	23 y	2 doses	H1
Jlu-10	Mvi/Songyuan.PRC/12.06/1	Songyuan	Outbreak	Throat swab	6 m	Nonvaccinated	H1
Jlu-11	Mvi/Changchun.PRC/12.06/13	Changchun	Outbreak	Throat swab	26 m	1 dose	H1
Jlu-12	Mvi/Changchun.PRC/10.06/9	Changchun	Outbreak	Throat swab	29 m	1 dose	H1
Jlu-13	Mvi/Changchun.PRC/11.06/14	Changchun	Sporadic	Throat swab	23 y	2 doses	H1
Jlu-14	Mvi/Songyuan.PRC/13.06/5	Songyuan	Sporadic	Throat swab	15 y	2 doses	H1
Jlu-15	Mvi/Changchun.PRC/16.06/20	Changchun	Outbreak	Throat swab	14 y	2 doses	H1
Jlu-16	Mvi/Jilinshi.PRC/50.06/27	Jilin	Outbreak	Throat swab	20 m	1 dose	H1

m month, *y* year

[a] The expanded program on immunization for measles consists of two doses vaccination in Jilin Province, one at 8 months old and the other at 7 years old

Sequence analysis

RT-PCR products were purified and sequenced using ABI PRISM™ 3730 DNA Sequencer, and then compared with the sequences of the Edmonston strain (GenBank: AF266288.1), the Changchun-47 (GenBank: EF033071.1) vaccine strain and the Shanghai-191 (GenBank: FJ416067.1) vaccine strain. The sequences of the wild-type strains were also compared with the WHO reference strains for genotype H1 and H2. Sequence data were analyzed using the Molecular evolutionary genetics analysis (MEGA) software version 4.0 [13], based on 450 nucleotides from C-terminus of the N gene and 537 nucleotides from the high variant area of the H gene (7877–8413 bp). The evolutionary distance was calculated by Kimura's two-parameter method and a phylogenetic tree was constructed using the neighbor-joining (NJ) method [14], based on nucleotide sequences of the N gene and the H gene. Each tree's reliability was estimated using 1,000 bootstrap replications.

Cross neutralization assay

To assess protective capacity of individual serum against both existing vaccine strains and newly isolated wild-type MV isolates, we collected 74 fresh serum samples from patients and healthy people in Jilin Province in 2010, including 14 patients with acute MV infection, 20 healthy infants pre-vaccination, 20 healthy infants post-vaccination of Changchun-47, and 20 healthy adults aged 20–40 years who had a history of measles vaccination or measles infection. Sera were tested by neutralization assays using Changchun-47 vaccine strain and two wild-type measles viruses designated Jlu-3 and Jlu-12 (see Table 1) as previously described [15].

Antigenic relatedness between the vaccines and wild-type MV strains was assessed as follows: immune sera were prepared in rabbits using vaccine strain (Changchun-47), Edmonston standard strain, and wild-type Jlu-3 and Jlu-12 viruses. Cross-neutralization assays were performed as described above. The antigenic ratio, representing antigenic differences between two viruses, was calculated using the formula of $R = (r1 \times r2)^{1/2}$, where R is the antigen ratio. r1 = (NT antigen titers serum A to antigen B)/(NT antigen titers serum A to antigen A) and r2 = (NT antigen titers serum B to antigen B)/(NT antigen titers serum B to antigen A), where NT is the neutralizing antibody titer [16, 17]. When R = 1–1.5 there is little or no antigenic differences between strains whereas R values of 1.5–2 indicate minor antigenic differences between strains. Significant antigenic differences are only obtained when R value is larger than 2.

Results

Epidemiology

During the years 2005 and 2006, the 16 MV isolates were collected from patients in six cities in Jilin province,

including six from Changchun, three from Jilin City, three from Siping, two from Songyuan, one from Yanbian, and one from Tonghua (Table 1). Among them, 12 were obtained from outbreak areas and four from sporadic cases. Their vaccination history showed that five patients were vaccinated with two doses measles vaccines, nine patients with one dose measles vaccine, and two patients (<8 months old) with non-vaccination. The 16 MV isolates were named using virus strain/city.country/week.year/isolate number nomenclature as previously recommended (Table 1) [18].

We analyzed the measles incidence and the MV vaccination coverage in China during 1986–2008 and in Jilin province during 2004–2008, according to the data published by Health Ministry of People's Republic of China (http://www.moh.gov.cn/publicfiles//business/htmlfiles/wsb/index.htm). It is found that the incidence of measles in China has been very low after 1986, and then increased since 2004, although programs to implement primary measles vaccination in infants have been intensive. Compared to the national average, the incidence of measles cases in Jilin Province was relatively low in 2004 but dramatically increased in 2006 in spite of high vaccination coverage.

To investigate the association of measles occurrence with age, we analyzed previously published data of measles cases, including the cases in this study, in Jilin Province during 2005–2006 (http://www.moh.gov.cn/publicfiles//business/htmlfiles/wsb/index.htm). The results showed that infants had the highest occurrence, and young children (aged 1–2 years) were also highly susceptible to measles. Notably, reported rates were also high in adolescents and young adults but no cases were reported in individuals more than 55 years old. Of all cases reported, 28.97 and 41.88% of measles cases occurred in children less than 2 years old in 2005 and 2006, respectively, compared to 42.72 and 46.13% of cases in those aged 10–40 years in the same calendar years.

Phylogenetic analysis

16 wild-type measles viruses were successfully isolated on Vero/SLAM from 82 throat swabs and 23 urine samples. PCR products derived from the COOH-terminus of the N and H genes were obtained from each of the 16 MV isolates and sequenced (Table 1). All of the 16 MV isolates in this study were clustered within genotype H1. Figure 1 shows the results of the phylogenetic analysis of carboxyl-terminal coding region of the N gene (a) and H gene (b) in the 16 MVs isolated in Jilin Province from 2005 to 2006 and the comparison with the Edmonston strain, the Changchun-47 and Shanghai-191 vaccine strains. Pairwise comparison within the 16 viral isolates showed that nucleotide variation was less than 1.3% in the N gene sequence and less than 1.89% in the H gene sequence. However, the nucleotide variation rose to 9.1–9.8% when N gene sequences were compared to the prototype Edmonston strain, and rose to 9.8–10.5% and 11.3–12.0% compared to the vaccine strains of Changchun-47 and Shanghai-191, respectively. Nucleotide variation in the H gene was 6.2–6.9% and 5.9–6.7%, compared to the two vaccine strains, Changchun-47 and Shanghai-191, respectively.

Interestingly, we found a mutation within the H gene of each of the 16 MV isolates at 38S → N (nucleotide 113G → A) that resulted in the loss of a potential glycosylation site. As a result, wild-type viruses recovered during the measles outbreak in Jilin Province possess hemagglutinins with only four potential glycosylation sites when compared to five potential sites described for Edmonston virus, Changchun-47 and Shanghai-191 vaccine strains [19].

Serological analysis and antigenic relatedness

Cross-neutralization assays showed that there was no statistical difference in the geometric mean neutralizing antibody titers between two wild-type virus strains, Jlu-3 and Jlu-12, when tested against all serum samples ($^{\blacktriangle}P > 0.05$, Table 2). Nonetheless, neutralizing antibody titers were significantly different when wild-type strains were compared with the measles vaccine strain (Changchun-47), where neutralization capacity of the sera against wild-type virus strains was statistically lower ($^{\blacklozenge}P < 0.05$, Table 2). In addition, among the four different groups of serum samples tested, post-vaccination sera from healthy infants showed the highest antibody titers when tested against the homologous vaccine strain (Changchun-47); however, the genetic mean titer (GMT) against wild-type strains was fourfold lower (Table 3). The capacity to neutralize wild-type strains was reduced to 1.90, 1.74, and 1.87 times when the GMTs of sera from acute measles cases, infant pre-vaccination and healthy adults were compared to Changchun-47 versus Jlu-3, respectively. The patients with acute measles had GMTs of 1:5.66 and 1:5.34 against Jlu-3 and Jlu-12 wild-type viruses, respectively, while the GMT against the vaccine strain was 1:10.77. These data suggest that the 16 patients from whom the 16 measles strains were isolated were susceptible to wild-type measles virus infection, perhaps resulting from the mutations of the wild-type measles virus.

We also examined the antigenic relatedness between the isolated wild-type strains and measles vaccines (Table 4). The antigenic ratio (R) between Jlu-3 and Jlu-12 was 1.14, which indicated no significant difference between the two wild-type strains. By contrast, we found that the antigenic relatedness between wild-type measles viruses and the existing vaccine strains was notably low, in which R values was 3.26 between Jlu-3 and Changchun-47, 5.62 between Jlu-12 and Changchun-47, 4.16 between Jlu-3 and Edmonston strain, and 6.07

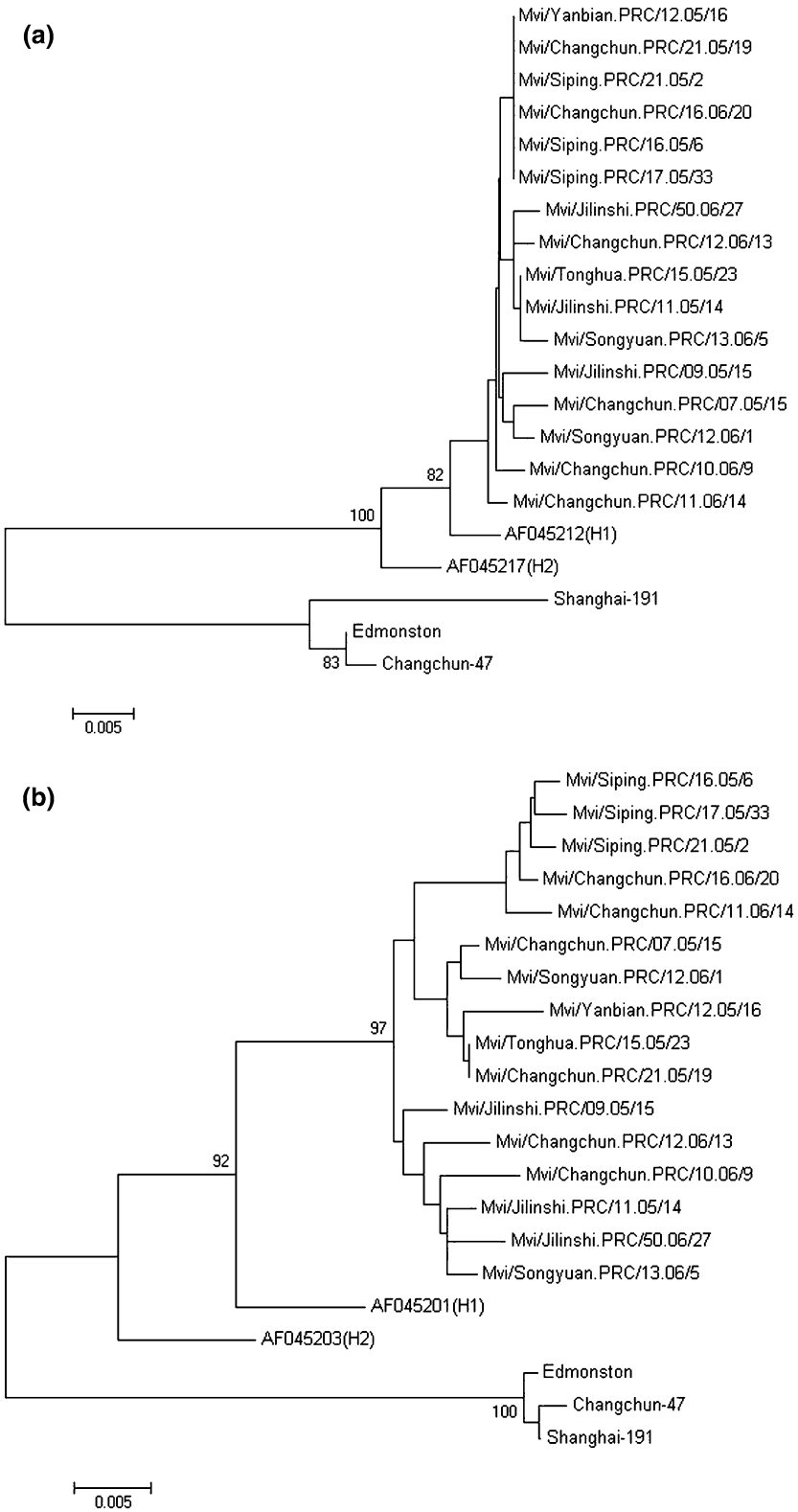

Fig. 1 Phylogenetic trees for the N gene (**a**) and the H gene (**b**) of MV strains. The 16 wild-type MV strains, Edmonston strain, H1, and H2 reference strains, Changchun-47 vaccine strain and Shanghai-191 vaccine strain were illustrated based on their nucleotide sequences. The evolutionary distance was calculated by Kimura's two-parameter method. The phylogenetic trees were constructed using the NJ method. The reliability of each tree was assessed by 1,000 bootstrap replications. *Numbers* at each branch indicate the bootstrap values of the clusters supported by the branch

between Jlu-12 and Edmonston strain, respectively. The results mean that circulating wild-type genotype H1 measles viruses recovered from the 2005 to 2006 outbreak in Jilin province are only 16–18% antigenically related to the approved vaccine strain and 24–36% antigenically related to the Edmonston strain.

Table 2 Comparison of neutralizing antibody titers against vaccine and wild-type strains in serum samples from acute measles cases, infants with pre- and post- measles vaccination and healthy adults

Serum	Cases	$\log_2$(1:NT)[a]		
		Changchun-47	Jlu-3	Jlu-12
Acute stage cases	14	3.43 ± 1.16◆	2.50 ± 0.52▲	2.42 ± 0.51
Infant prevaccine	20	4.45 ± 1.05◆	3.65 ± 1.27▲	3.50 ± 1.15
Infant postvaccine	20	6.05 ± 0.83◆	4.05 ± 1.32▲	3.90 ± 1.25
Healthy cases	20	4.75 ± 1.51◆	3.80 ± 1.40▲	3.75 ± 1.59

[a] *NT* neutralizing antibody titer

◆ Comparison of neutralization test results between vaccine stain Changchun-47 and wild-type virus Jlu-3 ($P < 0.05$)

▲ Comparison of neutralization test results between wild-type virus Jlu-3 and wild-type virus Jlu-12 ($P > 0.05$)

Table 3 Comparison of GMTs against vaccine and wild-type strains in serum samples from acute measles cases, infants with pre- and post-measles vaccination and healthy adults

Serum	Cases	GMT (1:)[a]			$\text{Ratio}^{b}_{(A)/(B)}$
		Changchun-47(A)	Jlu-3 (B)	Jlu-12	
Acute stage cases	14	10.77	5.66	5.34	1.90
Infant prevaccine	20	21.86	12.55	11.31	1.74
Infant postvaccine	20	66.26	16.56	14.93	4.00
Healthy cases	20	26.91	14.42	13.45	1.87

$\text{GMT} = 1 : (a_1^{-1} \times a_2^{-1} \times \ldots a_n^{-1})^{1/n}$, a titer of neutralizing antibody in the sera

[a] GMT (geometric mean titers) indicates the average level of antibody titers in a group

[b] $\text{Ratio}_{(A)/(B)}$, GMT of changchun-47 strain/GMT of Jlu-3 wild-type strain

Table 4 Comparison of antigenic ratios and the relatedness among different MV strains

Antigen	Changchun-47	Edm	Jlu-3	Jlu-12
Changchun-47	1.00	1.67 (60%)[a]	3.26 (31%)[a]	5.62 (18%)[a]
Edm		1.00	4.16 (24%)[a]	6.07 (16%)[a]
Jlu-3			1.00	1.14 (88%)[a]
Jlu-12				1.00

The antigenic ratio (R) represents the antigenic differences between two viruses (see "Cross neutralization assay" section)

[a] Antigenic relatedness was calculated using the formula of $1/R \times 100$

Discussion

In this study, we identified the epidemic MVs, which were responsible for the outbreak in Jilin Province during 2005–2006, as native genotype H1 wild-type strains. This is consistent with recently published data from China [20]. In addition, we identified genetic variations in both the N and H genes of the wild-type MV isolates obtained from this outbreak and demonstrated diminished neutralizing antibody titers when post-vaccination sera were tested against the wild-type strains. Therefore, the genetic alterations in genotype H1 MV isolates and the resulting antigenic changes may have contributed to an increase in the incidence of measles cases observed during this outbreak in a highly vaccinated population.

Vaccination symbolizes a great medical breakthrough pioneered by Edward Jenner over 200 years ago when he developed the first vaccine against smallpox [21]. In the twenty-first century, vaccination still remains the most effective means available for combating infectious diseases, including measles. Large-scale measles vaccination has led to a dramatic decrease in measles incidence and deaths worldwide [22]. Since measles vaccination was implemented in China in 1965, the estimated number of measles cases dropped from 2024.36/100,000 population per year during 1955–1964 to 97.25/100,000 in 1994 [23].

In the areas with high vaccination coverage, the incidence rate dropped further to less than 8/100,000 during 1995–2003 [24].

Nevertheless, measles continues to pose a substantial risk to the public health in China, with two outbreaks identified during 1986–1994 and 2005–2008 [1, 25]. As a result, the incidence rate in Jilin Province rose to 17.41/100,000 in 2006 [26]. It has been proposed that increased migration (floating population) and decreased vaccination may be responsible for the recent upswing in measles cases throughout China [20]. The preponderance of measles cases in susceptible individuals in the present report would support this. However, our findings suggest that antigenic variation in genotype H1 measles viruses may also be an important factor contributing to these outbreaks.

Measles virus is historically believed to be a single-serotype, thus currently circulating wild-type strains of measles should be antigenically similar to archived viruses. However, mounting evidence indicates that genetic variability occurs in wild-type strains, and existing vaccines may not be able to effectively protect populations from measles variants [1, 27, 28]. There are more incidences of measles in teenagers compared to younger children. It could be due to the mutation in wild-type strains, causing reduced protection of vaccination. Although there are currently two doses of measles vaccine applied in China (one dose at 8 months old and the other at 4–7 years), the antibody titer appears to decrease gradually. As a result, higher incidence would be expected in teenagers rather than younger children. In the early 1980s, Birrer and colleagues demonstrated the existence of MV variation based on the comparison between current circulating MV isolates and existing MV vaccine strains [29]. Measles viruses isolated in Liaoning Province, China during 1995–1997 showed genetic variation of 3.4–7.6% in the H gene sequences when compared to the Changchun-47 vaccine strain, while no differences were found when the H gene sequences were compared between MV isolates [30]. Another study reported that measles viruses isolated during an outbreak were almost identical in nucleotide sequence but genetically distinctive from the approved vaccine strain [31]. Similar findings were reported in other studies [32]. In agreement with these previous studies, here we found that wild-type MVs, although being genetically similar to each other, are constantly mutating with nucleotide aberration rates of 9.8–10.5% and 11.3–12.0% in the N gene and 6.2–6.9% and 5.9–6.7% in the H gene, when compared to the Changchun-47 and Shanghai-191 vaccine strains, respectively. In addition, our results further reveal that a mutation within the H gene results in the loss of a potential glycosylation site in each of the 16 wide-type isolates, which is consistent with previous reports [19, 33].

The antigenic variation of wild-type isolates has been further confirmed by our analysis by showing that two wild-type H1 strains were 88% related while, in contrast, H1 wild-type and genotype A vaccine strains were only 16 to 18% related antigenically (Table 4). Other investigators have also noted antigenic difference between vaccine strains and H1 genotype viruses; our data are consistent with a previous observation that some H1 MV isolates lacking neutralizing epitopes, could escape neutralization in the presence of monoclonal antibodies that bind the native epitope [34].

Changes in biological characteristics of measles virus may allow the virus population to rapidly adapt to new environments and escape host anti-viral responses [28]. To test this theory, we conducted cross-neutralization assays using serum samples collected from four different groups of individuals and compared their neutralization capacity against both wild-type and vaccine strains (Tables 2, 3). Our results reveal that although sera from children post-primary vaccination have the highest neutralization ability when tested against the homologous vaccine strain, the ability to neutralize wild-type strains was fourfold lower. In contrast, patients with acute measles had notably low antibody titers against both wild-type and vaccine strains, suggesting that the susceptibility to measles infection in these individuals was most likely due to the failure to vaccinate or primary vaccine failure. Our findings show that pre-vaccinated infants have low antibody titers, which could have originated from maternal antibody, against both wild-type and vaccine strains. However, increased incidence of measles in infants suggests that such low antibody titers could not protect infants against the wild-type strains.

Current strategies for tackling and eventually eliminating measles worldwide, having been set by WHO/United Nations Children's Fund (UNICEF), include increasing coverage of measles vaccine, enhancing effective measles surveillance, and offering two doses of vaccine to all children [22]. The specific goal of WHO in the Western Pacific Region, including China, was to eradicate measles by 2010 [35]. In China, the government has taken corresponding measures, and the measles vaccination coverage rate has been maintained at 97.4–98.6% since 2000, according to the data published by Health Ministry of People's Republic of China (http://www.moh.gov.cn/publicfiles//business/htmlfiles/wsb/index.htm). However, in spite of the high measles vaccination coverage, measles incidence rates have been rising in China over the last decade. In addition, there has been a shift in peak age of measles cases from young children to adolescents and younger adults [36]. To address these challenges, additional strategies are required to eradicate measles [37]. Our data show that there may be limitations in using current vaccines in targeting infections

caused by contemporary genotype H1 wild-type strains of measles virus. In addition to supplemental immunization with existing vaccines, there is also a need to consider the development of new measles vaccines as a surrogate strategy to achieve global measles eradication [38]. Moreover, the "second golden age" for measles vaccine may provide us a new insight for measles vaccine development [39].

Conclusions

Mutations in nucleotide sequences between contemporary genotype H1 measles viruses and approved vaccine strains may contribute to antigenic drift of measles virus. Our data suggest that the mutations in wild-type strains reduce the protection of vaccination while antigenic variation may lead to the escape from immune protection elicited by existing vaccines. Consequently, the incidences of measles increased. Whereas repeated vaccination enables individuals to be protected to some extend, thus strengthening current recommendations for supplemental immunization with existing vaccines. Moreover, diminished antigenic relatedness between vaccine and contemporary isolates strongly suggests a need for developing new measles vaccines to tackle circulating strains of wild-type H1 measles viruses more effectively.

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