Jpn. J. Infect. Dis., 71, 152–154, 2018

Short Communication

Ten-Year Surveillance of Measles Virus from 2007–2016 in Osaka City, Japan

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SUMMARY: Measles is a highly contagious infection caused by the measles virus (MV). This study performed long-term surveillance in order to survey the prevalence of MV. A total of 417 patients diagnosed with or suspected of having measles were tested for MV between January 2007 and December 2016 in Osaka City, Japan. Reverse transcription-polymerase chain reaction-based testing of clinical specimens showed that 54 patients (12.9%) were MV-positive. An MV epidemic occurred in 2007, in which all detected MV strains were genotype D5, an epidemic strain in Japan at that time. The detected wild-type MV strains in sporadic or outbreak-associated cases since 2011 included genotypes D4, D8, B3, and H1. Three vaccine strains (all genotype A) were also detected. Children <10 years of age accounted for 90.0% of the MV-positive patients in 2007. In contrast, adults ($\geq$ 20 years of age) accounted for the majority of MV-positive cases since 2011, as follows: 100%, 50%, 71.4%, 100%, and 87.5% of cases in 2011, 2013, 2014, 2015, and 2016, respectively. The recent high rate of two-dose MV vaccination coverage among children in Japan may have contributed to the reduced risk of MV infection and onset of measles in young persons.

Measles virus (MV) infects humans and causes measles. MV has a high infectivity and mainly causes exanthema, high fever, and catarrhal symptoms (1). About 30–40% of patients with measles develop complications such as diarrhea, otitis media, and pneumonia (2). In some cases, the infection may also cause encephalitis (2). After the development of the MV vaccine, the number of patients with measles decreased dramatically (3); the estimated numbers of deaths due to measles decreased from 651,600 in 2000 to 134,000 in 2015 (4). No MV epidemics have occurred in Japan since 2009. However, imported cases and related outbreaks have been reported (5–7). The World Health Organization (WHO) has a vision to achieve and maintain a world without measles (8). Japan was verified as having eliminated measles by the Measles Regional Verification Commission of the WHO Regional Office for the Western Pacific in March 2015 (9).

This study performed long-term surveillance for MV in the limited geographic area of Osaka City, the second largest city in Japan, with 2.7 million residents. The detected MV strains were analyzed molecularly to assess the transition of MV genotypes during the study period.

From January 2007 to December 2016, a total of 1,015 clinical specimens (whole blood, throat swab, and urine samples from most patients) were collected from 417 patients who were diagnosed or suspected of having measles in clinics and hospitals in Osaka City, Japan. Clinicians diagnosed measles according to the criteria defined by the Infectious Disease Control Law. Specimens were collected as part of a surveillance program conducted in Osaka City, Japan, and part of the national surveillance of viral infectious diseases in Japan following the Infectious Diseases Control Law.

Peripheral blood mononuclear cells and plasma were separated from whole blood specimens using Mono-Poly Resolving Medium (DS Pharma Biomedical Co., Ltd., Osaka, Japan) according to the manufacturer's instructions. Clinical specimens were tested for MV by performing nucleotide amplification tests. Between January 2007 and October 2015, MV RNA was detected through conventional nested reverse transcription-polymerase chain reaction (cnRT-PCR) targeting the 450 nucleotides of the nucleoprotein (N) gene. Viral RNA was extracted using a QIAamp Viral RNA Mini kit (QIAGEN Inc., Valencia, CA, USA). cDNA was synthesized using the PrimeScript RT reagent kit (Takara Bio Inc., Shiga, Japan). PCR and nested PCR were performed using PerfectShot™ Ex Taq (Takara Bio Inc.,) or EmeraldAmp® PCR Master Mix (Takara Bio Inc.,). The primer sequences (pMvGTf1m, pMvGTr1, pMvGTf2m, and pMvGTr2) for cnRT-PCR and PCR conditions were as described previously (10). Since November 2015, real-time RT-PCR was used to detect MV RNA using TaqMan Fast Virus 1 step Master Mix (Thermo Fisher Scientific, Inc., Waltham, MA, USA) on an ABI 7500 or StepOnePlus real-time PCR System (Thermo Fisher Scientific, Inc.). The conditions of the real-time RT-PCR were also as described previously (10) based on primer and probe sequences (MVN1139F, MVN1213R, and MVNP1163P) (11). When a sample was MV-positive on real-time RT-PCR, the N gene

Received July 20, 2017. Accepted October 31, 2017.
J-STAGE Advance Publication December 26, 2017.
DOI: 10.7883/yoken.JJID.2017.322

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Table 1. Measles virus detection and genotypes in Osaka City, Japan from 2007–2016

Year	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	Total
No. Tested patients/suspected	32	5	3	18	51	59	85	48	20	96	417
No. MV positives[1)]	20	0	0	0	1	0	2	14	1	16	54
Positivity rate (%)	62.5	0.0	0.0	0.0	2.0	0.0	2.4	29.2	5.0	16.7	12.9
Number of notified measles cases[2)]	n/a*	143	27	15	6	1	3	17	1	15	228
MV genotype (number of cases)	D5 (20)				D4 (1)		D8 (2)	A** (1) B3 (8) D8 (1) H1 (4)	B3 (1)	A** (2) D8 (3) H1 (10) NT*** (1)	
GenBank accession number(s)	AB720970 AB665392– AB665410				AB626984		AB809562 AB818353	B3 (AB931106– AB931110, AB971341– AB971343), H1 (LC002658– LC002661), D8 (LC015752)	LC128535	H1 (LC190473– LC190477, LC190479– LC190482, LC190484), D8 (LC190478, LC190483, LC190485)	

n/a*, Not applicable; A**, Vaccination derived; NT***, Not typed.
[1)]: Number of MV positives included MV genotype A (vaccine derived).
[2)]: Number of notified measles cases excluded patients positive for MV genotype A.

was amplified by cnRT-PCR. The 450 nucleotides of the N gene were sequenced and the genotypes were determined by phylogenetic analysis as previously described (10).

The results showed that 54 patients (12.9%) were MV-positive (Table 1). The MV detection and genotypes for each year were as follows: 2007 [D5 (n = 20)], 2011 [D4 (n = 1)], 2013 [D8 (n = 2)], 2014 [A (n = 1), B3 (n = 8), D8 (n = 1), H1 (n = 4)], 2015 [B3 (n = 1)], and 2016 [A (n = 2), D8 (n = 3), H1 (n = 10), Not typed (n = 1)]. During the study period, vaccine-induced strains were detected from 3 patients with MV genotype A in 2014 and 2016. When domestic epidemics occurred in 2007, all detected MV strains in Osaka City were genotype D5, a type indigenous to Japan. After the epidemic, no D5 strain was detected during the study period. Epidemiological data suggested that 2 MV-positive patients in 2011 [D4 (n = 1)] and 2013 [D8 (n = 1)] were imported cases because they had overseas travel histories and the identified genotypes matched those of the indigenous strains in their travel destinations (D4 from France and D8 from Indonesia). Genotype B3 suddenly appeared and increased in prevalence in 2014. Because this genotype had not been detected before 2014 in Osaka City during the study period, it was likely imported from another area in Japan or from abroad to Osaka City, where it subsequently spread. Of the 8 genotype B3-positive patients in 2014, 3 were from a single family and one had reported an overseas travel history to the Philippines. Genotype B3 was prevalent in the Philippines in 2014 (12). Another B3-positive adult patient reported visiting the hospital at the same time with one of the 3 B3-positive patients in their family. Another B3-positive infant (< 1 year of age) was believed to have been infected with MV through his father. No distinct epidemiological relationships were observed among the other MV-positive cases in 2014. One patient infected with MV genotype B3 in 2015 reported traveling to Indonesia. The number of genotype H1 MV-positive patients increased rapidly in August and September 2016. Five patients were working staff or users of Kansai International Airport. Another 3 patients positive for genotype H1 MV were medical service workers who had contact with the previously described patients infected with MV genotype H1. Therefore, this increase was due to the outbreak that occurred at Kansai International Airport (13).

The age distribution of the MV-positive patients is shown in Table 2. Patients < 20 years of age accounted for 90.0% of the MV-positive patients in 2007. In

Table 2. Age distributions of measles virus-positive patients in Osaka City, Japan from 2007–2016

Age (group)	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	Total
0	2							2			4
1	4						1			2[1)]	7
2–5	4										4
6–9	2										2
10–19	6							2[2)]			8
20–29	2				1			5		7	15
30–39							1	3	1	6	11
40–49								2			2
50–59											0
60–69										1	1
Total	20	0	0	0	1	0	2	14	1	16	54

[1)]: All two cases were genotype A.
[2)]: One of the two strains was genotype A.

contrast, patients ≥ 20 years of age accounted for the majority of MV-positive cases since 2008, at 100%, 50%, 71.4%, 100%, and 87.5% in 2011, 2013, 2014, 2015, and 2016, respectively. Excluding the MV-positive patients with genotype A (vaccine strain) in two 1-year-old patients in 2016, adult patients accounted for 100% of MV-positive-patients in 2016. Two-dose MV vaccinations are recommended for children in Japan, at approximately 1 and 5 years of age. A high rate of vaccination against MV at 1 year of age (96.2%) was reported in 2015 in Japan (14). The high rate of MV vaccination would contribute to maintaining the low incidence of MV-positive pediatric patients.

Despite achieving measles elimination, the high infectivity of the MV remains a public health threat in Japan. Once measles occurred, a secondary infection was reported to arise (15). Vaccination against MV is important for the prevention of infection, MV spread, and measles onset. Determination of MV genotype is also important to identify whether the detected MV strain is wild-type or vaccine-induced strain and to track MV transmission.

Acknowledgments The authors are grateful to the clinical doctors who participated in virus surveillance in Osaka City, Japan. This study was partially supported by JSPS KAKENHI Grant Number 26460819 to K.A.

Conflict of interest None to declare.

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