

Short Communication

An Outbreak of Measles Virus Infection due to a Genotype D9 at a Junior High School in Yamagata, Japan in 2004

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(Received October 14, 2004. Accepted November 29, 2004)

SUMMARY: We investigated a measles virus (MV) outbreak that occurred at a junior high school in Yamagata, Japan between January and February, 2004. We received throat swab specimens from three patients at this school and carried out virus isolation with Vero/hSLAM cells and virus genome detection by reverse-transcription polymerase chain reaction. As a result, we isolated the virus from one patient and succeeded in amplifying the MV genome from the others. Further sequence analysis of the N gene revealed that these viruses were completely identical, and that their genotype could be characterized as type D9, which has not been reported in Japan previously. We also identified D9 viruses in two students at other junior high schools in Yamagata. These results suggested that D9 strains were imported from a region outside Japan. The genotypes of MVs found in Yamagata have changed in recent years, with D5 predominating in 2001 and H1 predominating in 2002 and 2003 as reported as national surveillance data. Therefore, we should monitor carefully to be sure that D9 strains do not become the next predominant virus. The more the number of measles cases decrease, the more important become the roles of public health laboratories, which genotype MVs and monitor their circulation and transmission pathways.

Measles is an acute, highly contagious viral disease that affects mainly children and is characterized by fever, coryza, conjunctivitis, cough, and a specific enanthem followed by a generalized macropapular eruption (1). The routine reporting of suspected measles cases and laboratory testing of samples from patients is the backbone of the surveillance system (2).

According to the national epidemiological surveillance of infectious diseases in Japan, 33,812 measles cases were reported by pediatric sentinel clinics in 2001, which was the largest number for any year in the last decade. However, sporadic measles cases are still being reported in spite of a huge effort aimed at measles control by local public institutions (3).

Cases of measles virus (MV) have been reported by prefectural and municipal public health institutes to the Infectious Disease Surveillance Center at the National Institute of Infectious Diseases, Japan. Whereas the number of patients has decreased, reports of MV isolation have increased from 117 in 2001 and 62 in 2002, to 177 in 2003 (3). This virus strain survey revealed that in the 1990s the majority of MV isolates belonged to genotype D5, whereas genotype H1 strains have spread throughout Japan since around 2002 (3,4).

We here report a measles outbreak at a junior high school

in Yamagata Prefecture; the virus had a genotype D9, which has not previously been reported in Japan (3,5).

The junior high school is located in Yamagata city and the total number of students is 429. One case was defined as clinical measles based on a physicians' diagnosis. According to the epidemic curve (Fig. 1), the total number of cases was 28 (attack rate [AR]: 6.5%) (Table 1). The index case was supposed to have developed symptoms on January 21st, 2004 and the outbreak continued to the end of February, 2004. Six of the infected students were admitted to hospitals for treatment (21.4%). All the hospitalized cases were unvaccinated students. The age distribution of the cases was from 12 to 15 years old, and the male:female ratio was 20:8. Fifty percent of the infected students were in the 2nd grade. Other observations on the students' activities were as follows: (i) the classrooms for the 3rd grade students were apart from the others, (ii) the 3rd grade students had already finished their athletic seasons at the time of the outbreak, (iii) most of the extracurricular activities were carried out separately by the male and female students; and (iv) no school assembly involving the whole student body had been held since the beginning of January due to an influenza outbreak. An examination of the vaccination history showed that 18 of the 28 infected individuals had been vaccinated (64.3%). The MV vaccines used were all single-dose MV or measles, mumps, and rubella (MMR) vaccines. The AR among unvaccinated students (ARU) was 31.3% (10/32) and the AR among the single-dose MV or MMR vaccinated students (ARV) was 4.8% (18/377) according to data obtained by questionnaire. A basic estimate of vaccine efficacy (VE) was calculated

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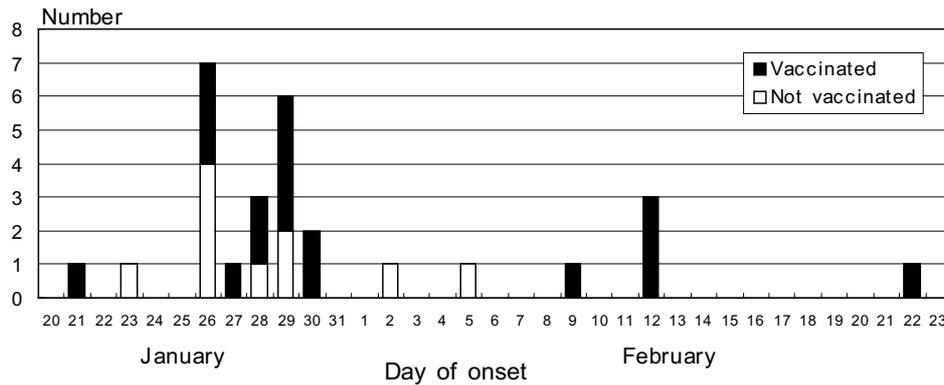


Fig. 1. Epidemic curve of measles cases among the students at a junior high school, Yamagata, Japan, Jan. - Feb., 2004 ($n = 28$). The days of onset were estimated based on clinical symptoms.

Table 1. The number of the measles patients in each grade at a junior high school in Yamagata, Japan in 2004

Grade	No. of patients	Total	Attack rate (%)
1st	12	130	9.2
2nd	14	157	8.9
3rd	2	142	1.4
Total	28	429	6.5

using the following formula: $VE (\%) = \{(ARU - ARV) / ARU\} \times 100$. Using this formula, the VE was estimated to be 84.7% for the 429 students. The above data indicate that the outbreak occurred mainly among the unvaccinated, although there were also some cases of secondary failure. During this outbreak period, one to five sporadic cases were also reported from each of 13 elementary, junior high, and high schools in and around Yamagata city.

For virus isolation, we received throat swab specimens from three students at the index school. The specimens were collected on January 28th and February 13th and 14th, respectively. We also received two throat swab specimens from two students at two other junior high schools, who were clinically diagnosed with MV infection in January and February 2004, respectively. After centrifugation at 3,000 rpm for 15 min, we inoculated these five specimens on Vero/hSLAM cells, which were kindly provided by Prof. Y. Yanagi, Department of Virology, Faculty of Medicine, Kyushu University (6).

We also attempted to amplify and detect the MV genome by reverse-transcription polymerase chain reaction (RT-PCR). Briefly, RNA was extracted from 200 μ l of each specimen using a High Pure Viral RNA Kit (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's instructions. Complementary DNA was synthesized by RT reaction and PCR consisting of 40 cycles of 94°C for 30 sec, 55°C for 30 sec, and 72°C for 60 sec was carried out as described previously (7). Two primer pairs, pMvGTf1 and pMvGTr1 for the 1st PCR, and pMvGTf2 and pMvGTr2 for the nested PCR, were used (8). For sequencing analysis of the N gene, the Gene Rapid system (Amersham Biosciences Corp, Piscataway, N. J., USA) was used according to the manufacturer's instructions with the same primers as for PCR. For further PCR and sequence analysis of the H gene, we used primers MVH-IF, MVH-IR, MVH-BF, MVH-DF and MVH-FF (9).

As a result, we isolated two MVs, one from a student at

the index school (MVi/Yamagata.Jpn/5.04) and one from a student at another school (MVi/Yamagata.Jpn/3.04). We also succeeded in detecting the MV genome in the three patients (MVs/Yamagata.Jpn/7.04/1-3) from whom we failed to isolate the virus. Therefore, we were able to identify the MV infection in all five cases investigated.

The sequence data of the N gene revealed that the 456 bps region analyzed was identical among the five cases in 2004 (GeneBank Accession No. AB186905 – AB186909). These results indicated that identical MVs caused outbreaks at several (at least three) junior high schools around Yamagata city between January and February 2004. A search of the BLAST database showed that the nucleotide sequence was most similar to the MVs/Vic.AU/21.99 strain (98.0% identity), which was recently designated as genotype D9 from Australia (9,10). The phylogenetic tree confirmed that the MV isolates in Yamagata in 2004 belonged to the same branch as the reported D9 strains (Fig. 2). This D9 genotype has so far been found only in Indonesia, Venezuela, Colombia, and Australia (5,10,11). Analysis of the sequence of the H gene from the two MV isolates (MVi/Yamagata.Jpn/3.04 and MVi/Yamagata.Jpn/5.04) confirmed that these strains could be characterized as the D9 genotype (Accession No. AB186910 and AB186911).

The global laboratory network of the World Health Organization (WHO) has two functions in measles surveillance. The first is monitoring and verifying virus circulation and the second is assisting in the determination of the measles susceptibility profile of a population in a specific situation (2). In terms of monitoring, analysis of the chronological and geographical change in circulating genotypes enables us to determine the epidemiological linkage and transmission pathways between the measles cases and outbreaks. Internationally, even the importation and exportation of MVs based on the global distribution of genotypes have been discussed (4,5,9,11,12). In this sense, the present isolation of genotype D9 strains in Yamagata might suggest that they were imported from a region in which genotype D9 strains had been circulating previously, as described above. The genotypes of MVs found in Yamagata have changed in recent years, with D5 predominating in 2001 and H1 predominating in 2002 and 2003 as reported as national surveillance data, as shown in Fig. 2 (3,4). Therefore, we should monitor carefully to be sure that D9 strains do not become the next predominant virus.

In order to control MV infections, an increase in vaccination coverage is of primary importance (3). At the same time, public health laboratories will play an increasingly

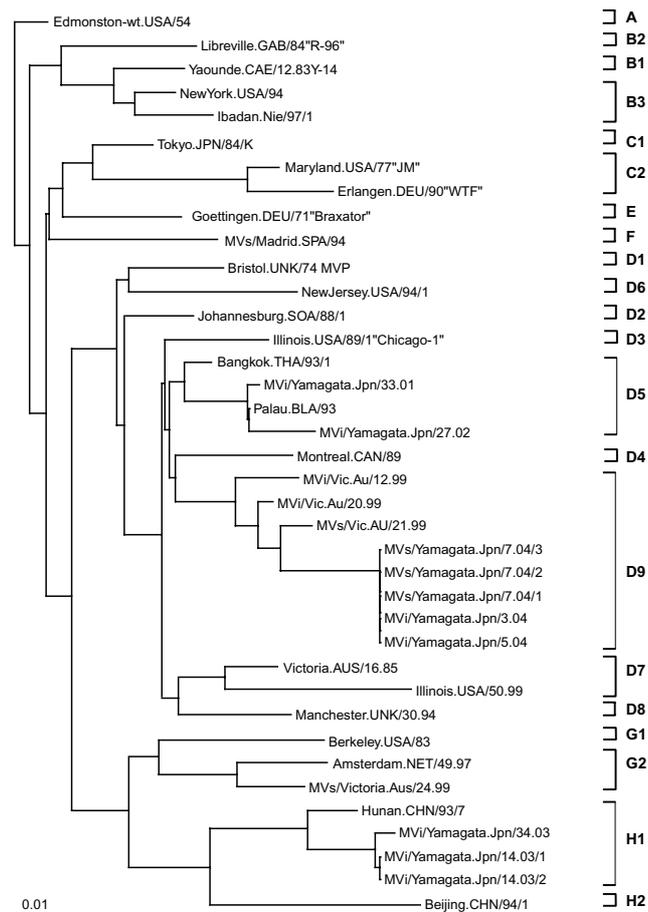


Fig. 2. Genetic relationship between measles viruses isolated in Yamagata, Japan, in 2001-2004 (GeneBank Accession No. AB186900-186909) and the reference strains established by the World Health Organization (10). Phylogenetic tree was based on the nucleotide sequences (456 bps) coding for the carboxy terminal end of the nucleoprotein and was generated by the CLUSTAL W program. The scale indicates 1% nucleotide differences.

important role in measles surveillance (2). As part of this surveillance effort, we have herein reported the first case of an MV outbreak in Yamagata due to genotype D9.

ACKNOWLEDGMENTS

We thank Prof. Y. Yanagi, Department of Virology, Faculty of Medicine, Kyushu University, for providing the Vero/hSLAM cells. We also thank the medical staff and the residents of Yamagata Prefecture for their assistance in the surveillance of viral infectious diseases.

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