Jpn. J. Infect. Dis., 61, 2008

Laboratory and Epidemiology Communications

Isolation of Measles Virus Classified as D5 Genotype during an Outbreak in Kobe City, Japan, in 2007

Kyoko Akiyoshi*, Tomoko Suga, Tsunekazu Haruta[1], Kenji Yoshida, Ryusuke Taba, Masaru Yamakawa, Michi Kawamoto, Mari Shibata[2] and Masae Itoh[2]

Kobe Institute of Health; [1]Kobe City Medical Center General Hospital, Kobe 650-0046; and [2]Nagahama Institute of Bio-Science and Technology, Shiga 526-0829, Japan

Communicated by Ichiro Kurane

(Accepted October 10, 2008)

Measles became widely prevalent in Japan in the spring of 2007 after an interval of 6 years (1). The reported number of measles cases started to increase in Kobe City at the beginning of May. The Health and Welfare Division of the Kobe City Government intensively implemented the measles surveillance. From May to December 2007, 155 patients aged 15 years or over and 100 patients aged under 15 years were reported to the Kobe Public Health Center as child and adult cases, respectively (Table 1).

Peripheral blood and throat swab specimens were collected for virus isolation. Lymphocytes separated from peripheral blood specimens and throat swabs were inoculated with B95a cells (2). The development of multinucleated giant cells due to cell fusion, the characteristic cytopathic effect (CPE) of measles virus (MV), was checked everyday as a sign of virus isolation. Virus isolation was confirmed by indirect immunofluorescent test using antibody specific to MV. A total of 55 isolates were obtained from 41 patients; 40 isolates from lymphocyte specimens and 15 from throat swab specimens (Table 1). MV-isolation rates were higher with peripheral blood lymphocyte samples (56%) than with throat swab specimens (24%). The results suggest that peripheral blood samples are preferable as specimens for MV isolation.

The time required for virus isolation in B95a cell culture was compared between vaccinated and non-vaccinated patients. The CPE characteristic of MV was observed at 0.9 ± 0.5 days after inoculation in specimens from non-vaccinated cases, with the earliest detection at 14 h after the inoculation, while CPE was observed at 3.1 ± 2.3 days in those from vaccinated patients. All 41 patients from whom MV was isolated demonstrated fever and rash as clinical symptoms except for one vaccinated patient who did not

Table 1. Measles virus isolated in Kobe City from May to December in 2007

Month	No. of reported cases		No. of cases[1)]		No. of virus isolates[2)]	
	Age		Age		Peripheral blood	Throat swab
	≧15	<15	≧15	<15		
May	26	3	7 (9)	1 (1)	7 (9)	4 (8)
June	43	19	7 (14)	0 (6)	7 (19)	2 (17)
July	16	3	3 (4)	0 (2)	3 (6)	1 (4)
August	4	8	0 (0)	2 (3)	2 (3)	1 (2)
September	10	6	1 (2)	1 (3)	2 (4)	1 (4)
October	33	13	5 (10)	2 (6)	7 (14)	3 (15)
November	15	29	5 (6)	2 (4)	7 (10)	0 (3)
December	8	19	1 (1)	4 (8)	5 (7)	3 (9)
Subtotal	155	100	29 (46)	12 (33)	40 (72)	15 (62)
Total	255		41 (79)		55 (134)	

[1)]: Measles cases confirmed by virus isolation. No. of cases from whom specimens were collected is shown in parentheses.
[2)]: No. of collected specimens is shown in parentheses.

*Corresponding author: Mailing address: Kobe Institute of Health, 4-6 Minatojima-nakamachi, Chuo-ku, Kobe 650-0046, Japan. Tel: +81-78-302-6252, Fax: +81-78-302-0894, E-mail: kyoshi@mrg.biglobe.ne.jp

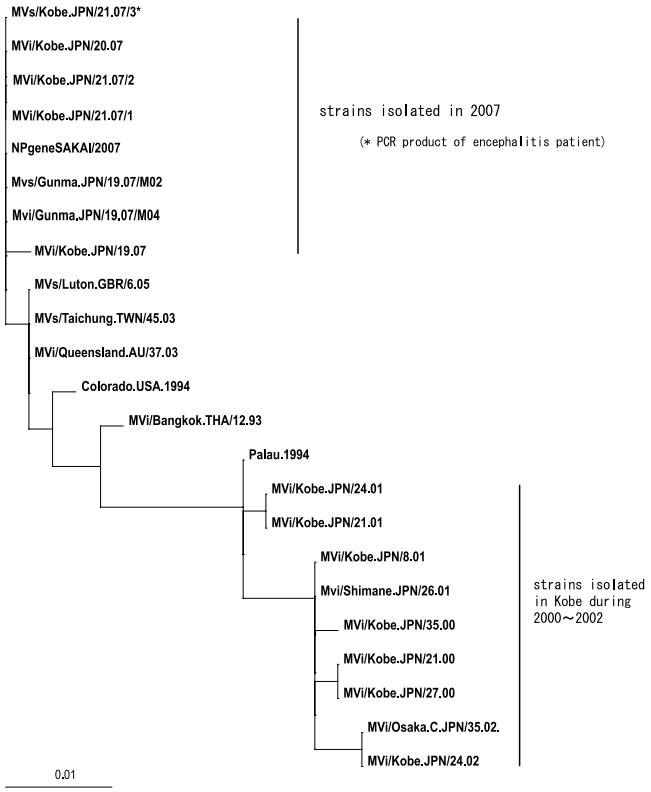

Fig. 1. Phylogenetic tree according to the nucleotide sequences of measles virus (MV) strains that belong to D5 genotype. Sequences of 456 nucleotides at the 3′ terminus of the N gene were compared. The distance was calculated using Kimura's correction. The tree was plotted by the neighbor-joining method using ClustalW of the DNA Data Bank of Japan (DDBJ).

develop rash. Catarrhal symptoms like cough were observed in 50 and 88% of the vaccinated and non-vaccinated patients, respectively, suggesting that the clinical symptoms of the vaccinated patients were comparatively mild.

For genetic analysis, a 456-nt fragment at the 3′ terminal of the N gene was amplified by reverse transcription-polymerase chain reaction (RT-PCR) from B95a cells infected with each of 4 isolates and also directly from a throat swab from a case with encephalitis. The sequences were determined. One nucleotide difference was detected between the MVi/Kobe.JPN/19.07 isolate and the other 4 isolates including the one from the encephalitis case. All the MV isolates were assigned to genotype D5 based on a comparison with other MV strains listed in the GenBank databases of the National Center for Biotechnology Information (NCBI). The phylogenetic analysis indicates that these D5 isolates were from the same strain as those isolated in other regions of Japan in 2007, but were considerably different from those isolated in Kobe City from 2000 to 2002 (Fig. 1). These new D5 isolates were very close to MVs/Luton.GBR/6.05, MVs/Taichung.TWN/45.03, and MVi/Queensland.AU/37.03, suggesting the possibility that these isolates were introduced into Japan from foreign countries.

The outbreak of measles has continued in Kobe in 2008. The establishment of an efficient surveillance system is thus strongly required to foster closer interaction between laboratories and hospitals.

This article appeared in part in the Infectious Agents Surveillance Report (IASR), vol. 29, p. 17-19, 2008 in Japanese.

REFERENCES

1. National Institute of Infectious Diseases and Tuberculosis and Infectious Diseases Control Division, Ministry of Health, Labour and Welfare (2007): Measles, Japan, 2006-2007. Infect. Agents Surveillance Rep., 28, 239'-240'.
2. Kobune, F., Sakata, H. and Sugiura, A. (1990): Marmoset lymphoblastoid cells as a sensitive host for isolation of measles virus. J. Virol., 64, 700-705.