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Genotype Analysis of Measles Viruses, 2002

Abstract

In 2002, through the communicable disease reporting system, 77 suspected cases of measles had been reported. Of them, 27 were confirmed by serological testing. By conventional epidemiological investigation, they could be grouped in five chains and 13 sporadic cases. The Taichung area had the largest number of 15 confirmed cases. This was second to the 31 confirmed cases identified in 1994. The first confirmed case in 2002 occurred in February. In July and August, cases were reported one by one in the Taichung area; and in mid-September till early October, cases were still reported from three junior high and primary schools in Taichung County. The outbreak was controlled in late-October after the checking of immunization records and make-up vaccination by the Disease Control Division of the Center for Disease Control. In the outbreaks in schools, wild-type measles viruses were isolated in specimens of three patients. By nucleoprotein gene sequencing, they were found to be the H1 genotype. Conventional epidemiological surveys of contact history could not always disclose associations between cases. In addition to the isolated virus strains, RNAs were extracted from clinical specimens

such as sera, throat swabs or urine for RT-PCR. The products so obtained were sequenced, and compared for their nucleoprotein genes to investigate the epidemic from the viewpoint of molecular biology.

Introduction

Measles is an acute highly communicable viral disease. Prior to widespread immunization, measles was an essential communicable disease of childhood. In Taiwan, since the immunization of MMR for all children one-year and three-month old since 1992, with the exception of a small-scale outbreak in a kindergarten in Taoyuan County in 1994, in the ten-year period between 1992 and 2002, only a few confirmed cases were reported each year, and most of them were children younger than nine months old who had not been immunized the first dose yet.

Measles is a single-ply RNA virus of the family *Paramyoviridae*. Symptoms are coughing, coryza, conjunctivitis (the 3C's of measles infection), fever, and maculopapular rash. Under poor sanitary conditions and nutritional status, the fatality is 10%. Likely complications are giant cell pneumonia, inclusion body encephalitis, and subacute sclerosing panencephalitis. Since the introduction of the live attenuated vaccine, infections have been greatly reduced. The World Health Organization has thus targeted its eradication after polio.

Measles virus has only one serotype. The wild-type measles viruses, however, are relatively different in their genotypes. In 1998, the WHO officially declared the nomenclature principles for measles viruses, using gene-sequencing comparison of haemagglutinin-H and nucleoprotein-N as bases for the typing of wild-type measles viruses. Of the six component proteins of measles viruses, H and N genes show the greatest difference of as

high as 7%. The variability of the genetic compositions of the 150 amino acids at the end of COOH nucleoprotein in different virus strains can be as high as 12%[(1)]. This was the gene sequence used in the present study for typing. By the global distribution of already isolated wild-types of measles viruses, there seems to be geographical differentiation[(2)]. By comparing the sequences of the present study with the gene sequences of reference virus strains announced by the WHO, and taking into consideration data of conventional epidemiological investigations, it was possible to understand to some extent the epidemiology of measles in the Taiwan area in 2002.

Materials and Methods

Specimens

Sera, whole blood containing anticoagulant, urine or throat swabs from suspected cases meeting the definition of reporting (two of the symptoms of fever, coughing, coryza and conjunctivitis, and rash) in their acute period seven days after onset were collected and sent under low temperature to the Respiratory Tract Virus Laboratory of the Center for Disease Control for virus culturing, isolation and molecular biological testing.

Pre-Treatment of Specimens

1.Throat swabs were placed in 2 ml DMEM culture medium containing 2x antibiotics (200 unit/ml penicillin, 200 ug/ml streptomycin) and mixed. The throat swabs were removed after one hour, and the fluid was used for inoculation.

2.2 ml of the blood containing anticoagulant was mixed with 2 ml HBSS, poured slowly into the upper end of the centrifugal tube containing 3 ml Ficoll-Paque, centrifuged at 400 xg, 18℃-20℃ for 40 minutes. An aseptic pipette was used to suck from the obscure area (lymph corpuscles) between

the Ficoll-Paque and the serum, sucked three times by adding three-time volume of HBSS, washed thoroughly, and centrifuged at 100 xg, 18℃-20℃ for 10 minutes, removed the upper fluid, washed again, and mixed with 2 ml of DMEM culture medium containing 2x antibiotics. The fluid was used for inoculation.

3.Urine was centrifuged at 1,500 rpm, 4℃ for 10 minutes, removed the upper fluid, and the sedimentation was mixed thoroughly with 2 ml of DMEM culture medium containing 2x antibiotics, the fluid was for inoculation.

Culturing of Viruses

B95a cell strain was used for the culturing of viruses[3]. B95a medium was placed in a 25T tube, inoculated with 0.5 cc of the pre-treated specimen, shaken thoroughly, and placed under 37℃, in 5% CO_2 culture box for one hour, added DMEM culture medium containing 2% FBS and 1x antibiotics (maintenance medium), placed under 37℃, 5% CO_2 culture box. They were observed every day for cytopathic effects (CPE). B95a cells grew fast; if no CPE was seen after two days, repeated the culturing. Culture medium was sucked, placed in Trypsin-EDTA under room temperature for one minute. The cells were loose. They were mixed with DMEM thoroughly, placed evenly in two 25T culture tubes, added 5 ml of culture medium, placed under 37℃, 5% CO_2 culture box, and observed for CPE each day. If not, repeated culturing after 3-4 days. If no CPE was seen, it was decided negative, and testing terminated. If there were CPE's, waited until they reached 70%, removed them from culture tubes, placed on slides for fluorescent dying assay. The remaining cell fluid was kept separately in refrigerator under -80℃.

Immunofluoresent Assay

Inoculated cells were removed from culture bottles, centrifuged at 3,000 rpm, 4℃ for 15 minutes. The upper suspension fluid (for molecular

biological testing) collected, placed in PBS resuspend. 10 ul of it was taken, placed in 21-hole slides, and when dried, put in test reagent containing -20℃ acetone for 10 minutes, and when dried, performed immunofluorescent assay by the commercialized Measles IFA kit (Light Diagnostic Measles-IFA kit, Chemicon International, Inc., catalogue-3187) in the following way: measles monoclonal antibody was put in each well; put the slides in moisture chamber under 37℃ for 30 minutes; washed with PBS/Tween 20 and rinsed slides for 10-15 seconds, dried, added anti-mouse IgG/FITC conjugate, put in moisture chamber under 37℃ for 30 minutes, repeated slide rinsing, dried, and added mounting fluid, and covered with slides. Slides were observed with microscopes. Cells with apple-green fluorescent color were measles virus positive.

Serological Testing

The commercialized Enzygnost Anti-Masern-Virus/IgM&Enzygnost Anti-Masern-Virus/IgG (Dade Behring, Marburg, Germany) was used for testing of IgM and IgG antibodies in serum or plasma. 20 ul of specimen was taken, added 400 ul sample diluent to dilute to 1:21. For IgM testing, 200 ul (1:21 dilute) serum was added 200 ul RF Absorbent (final 1:42 dilute) for 15 minutes (to remove rheumatoid factor and IgG in serum to avoid false positive); 150 ul of reaction fluid was collected and placed in 2-hole plate. The antigen in one hole was permanent siman kidney cell for the culturing of measles virus; in another hole was permanent siman kidney cell as control antigen. For IgG reaction, 200 ul of sample diluent added on the 2-hole plate; collected 20 ul (1:21 dilute) serum, added on the 2-hole plate (final 1:231 dilute), placed under 37℃ for one hour, washed, added IgM&IgG conjugate, placed under 37℃ for one hour, washed again, added substrate under room temperature for 30 minutes, added stop solution to

terminate reaction. Read the light absorbing value under 450 mm wavelength; positive if the value was >0.2, and negative if the value was <0.1.

Molecular Biological Testing

Extraction of RNA

QIAamp Viral RNA Kit of QIAGEN was used for the extraction of RNA. 140 ul of serum, throat swab, urine and virus fluid specimens was taken and added 560 ul Buffer AVL under room temperature for 10 minutes, added 560 ul of absolute alcohol to mix thoroughly. The mixture was put through QIAamp spin column, washed with Buffer AW1&AW2, and resolved RNA with pure water. The RNA so prepared was used for RT-PCR (Reverse Transcription Polymerase Chain Reaction). Primers were chosen with reference to published literature[(4)] and experiences of laboratories. The nucleic acid sequence corresponding to the 150 amino acid at the N-protein COOH end was used. To improve sensitivity, product of the first RT-PCR was used again for nested PCR testing.

Nested RT-PCR

（1）Reverse transcriptase

5 ul of virus RNA was taken, added 50 ul of MuLV-reverse transcriptase containing 1x PCR buffer (50 mM KCl, 10 mM Tris-HCl), 5 mM $MgCl_2$, 1 mM ddNTPs, RnaseOut 20 U, and 0.5 uM of antisense primer MV64 (MV64:5'-TAT ACC AAT GAT GGA GGG TAG-3') to a final volume of 20 ul, placed under 42℃ for 15 minutes, 99℃ for 5 minutes, and 4℃ for 5 minutes.

（2）Polymerase chain reaction (PCR)

CDNA collected from the reverse transcription was conducted PCR. 20 ul of cDNA collected from the above procedure was added 50 mM KCl, 10 mM Tris-HCl, 2.0 mM $MgCl_2$, and primers MV59 (MV59: 5'-GAT ATG

TGA CAT TGA TAC ATA TAT-3') 1- pmole, added 2.5 units of Taq polymerase (to a total volume of 100 ul), denatured at 94℃ for 2 minutes, reacted at 94℃ for 30 seconds, 50℃ for 30 seconds, 72℃ for 1 minute for 35 times, and finally reacted at 72℃ for 7 minutes.

（3）Nest PCR

The augmented products of the first PCR were used for nest PCR. 3 ul of DNA was taken, added 25 ul of 2x PCR Master Mix (MBI FERMENTAS 2x PCR Master Mix) and 5 pmole each of primers MV60 and MV63 (MV60: 5'-GCT ATG CCA TGG GAG TAG GAG TGG-3'; MV63: 5'-GGC CTC TCG CAC CTA GTC TAG-3'), added water to a total volume of 50 ul, denatured at 94℃ for 2 minutes, reacted 35 times at 94℃ for 30 seconds, 60℃ for 30 seconds, 72℃ for 1 minute, and finally at 72℃ for 7 minutes.

Agarose Gel Electrophoresis and Phylogenetic Tree Analysis

The product was electrophoresised with 1.5% agarose gel at 100 V for about 30 minutes, removed and dyed with ethidium bromide for 5 minutes, washed and read with the developing system. Positive electrophoresis after being augmented by nest-PCR would show on the agarose gel a product of 580 bps. After the PCR product was sequenced (for sequencing procedures, refer to [5]), a 456 bps of nucleic acid was collected for matching with the WHO measles genotype reference strain (see Table 1). Molecular Evolutionary Genetics Analysis (MEGA) version 2.1 soft was used for phylogenetic analysis by "neighbor-joining" method and bootstrapped for 1,000 times.

Results

In 2002, there had been 27 serologically confirmed measles cases. In

urine specimens of two of them and whole blood of one of them, measles viruses were isolated in their blood cells. For ages of the 27 measles cases, their dates of rash appearing, dates specimens collected, kinds of specimens collected, immunization status, results of serological testing, and naming of genome sequencing, see Table 1.

Nucleic acid was extracted from the isolated virus strains and other specimens (urine, sera, throat swabs) that were serologically confirmed but not isolated viruses. They were augmented with MV64 and MV59 primers, and augmented again by nest-PCR with MV60 and MV63 primers. Their electrophoresis showed 580 bps section as Figure 1. When the nest-PCR product was purified and sequence-analyzed with AB1377, as Table 1, 13 of the 23 cases showed molecular biological sequencing. When the 456 bps sequence at the end of COOH was collected for comparing the similarity of nucleic acid (Figure 2), the similarity was found to range from 88.6 to 100%. The similarity between MVs/Taichung.TWN/10.02 and MVs/Kaohsiung. TWN/16.02 was 96.5%; that with other eleven strains ranged from 88.6 to 92.3%. The similarity between the rest 11 strains other than MVs/ Taichung.TWN/10.02 and MVs/Kaohsiung.TWN/16.02 was 94.3-100%. The five sequences that had similarity of 100% were MVs/Taichung. TWN/38.02, Mvi/Taichung.TWN/40.02/1, MVi/Taichung.TWN/40.02/2, MVi/ Taichung.TWN/40.02/3, and MVs/Hisnchu.TWN/40.02/4. They were geographically related. Of them, MVs/Hsinchu.TWN/40.02/4 though was reported and collected in the Hsinchu area, the case lived in Taichung and worked in Hsinchu. The case could have been associated with the three cases reported in the same week.

The N genome sequence of the WHO reference measles genotype strain

found on the NCB1 web site (Table 2)[(1)] and the sequences obtained in the laboratory were analyzed for phylogenetic tree. A piece each of 456 bps was taken and analyzed by MEGA version 2.1 with the "neighbor-joining" method, and bootstrapped for 1,000 times. The findings are shown in Figure 3. It was found that the three virus strains isolated in 2002 were all of the H1 genotype; the other sequences augmented directly from specimens showed D3 and D5 genotypes.

Discussion and Conclusion

In 1998, the WHO announced the nomenclature principles for measles viruses[(1)]. Basically, MVi refers to virus strains cultured through cells, and MVs refers to sequences obtained through virus RNA analysis from clinical specimens. Other features concerning virus isolation strains/virus sequences are location where the case occurs, such as a state or province (in the present case, name of county/city is used to indicate the geographic location), country, the time virus is collected (week/year), and numbered if two viruses appear in the same week, and the genotype of virus at the end (must at least base on the N gene of the 450 nucleic acid at the COOH end), and other remarks such as complications of measles, including inclusion body encephalitis – MIBE, subacute sclerosing panencephalitis – SSPE. MVi/Taoyuan.TWN/24.94/1 [H1], for instance, stands for the first measles virus isolated in Taoyuan on the 24th week of 1994, and of H1 genotype. MVs/Hualian.TWN/17.03[G3]SSPE stands for the genome sequence isolated in Hualian directly from specimen of a SSPE patient on the 17th week of 2003.

By data of the conventional epidemiological investigations, contacts between cases were grouped in either the chain or sporadic cases. By this principle, the 27 cases in 2002 came under five chains and 13 sporadic cases.

It can be noted from Table 1 that, by epidemiological investigation, chain one and chain four were cases of mother-to-child transmission. By the date of rash appearing, mother became infected first, and then transmitted the infection to children. Of them, case MVs/Pingtung.TWN/33.02 became ill two weeks after her return from the mainland China, and thus was considered an imported case. Her genotype analysis showed that it was an H1 type, meeting the WHO information that distribution of measles genotypes has certain geographic distinction (Table 3)[(2)]. In another analysis, the D3 genotype case (MVs/Taichung.TWN/10.02) that became ill two weeks after return from the Philippines also illustrated the point. Chain two and chain five were infections between classmates. In chain three, a child became infected at home, and then transmitted the infection to two siblings and a classmate.

By the molecular biological monitoring of viruses, except chain one, though no routes of direct transmission could be traced in chain two through chain five, the similarity of the virus sequences and the fact that they were all of H1 genotype, and also their geographic distribution, and that their onset concentrated in two incubation periods, all indicated that they were epidemiologically associated[(6)]. From the nucleic acid in Figure 2, the similarity of 100% in the genome sequences between chain two (MVs/Taichung.TWN/38.02), chain three (MVi/Taichung.TWN/40.02/1, MVi/Taichung.TWN/40.02/3), and chain five (MVi/Taichung.TWN/40.02/3) was found. Again, for their close localities, they should be associated. By the epidemiological data, in early September, a 26-old woman became ill after return from the mainland China (Table 1, No. 49/02). Gene sequencing was not possible for delay in the collection of specimen from her (14 days after rash appeared). By the locality and date of onset, it

could be speculated that she was the source of all later infections in the Taichung area. Though the conventional epidemiological investigation was unable to trace the history of contacts, the possibility of viruses spreading around could not be overlooked[7].

One case of D5 (MVs/Kaohsiung.TWN/16.02) genotype was hard to explain. By the distribution of wild-strain measles viruses, D5 genotype is geographically from Japan, Thailand and Namibia. Epidemiological investigation did not find any overseas travel history of the case. Data of the measles virus strains in Taiwan before the mass immunization are not available. It was not possible to speculate that the case was either an indigenous type or was infected by a source of infection overlooked or untraceable during the course of investigation.

Measles control in Taiwan began with the universal MMR immunization of children 15 months old in 1992. Ever since, the three-year cycle epidemic has not been seen. What is noticeable is, children born in the period between the measles vaccine was brought into Taiwan (1967) and the mass immunization began (1992) when measles was somewhat controlled by the intervention of vaccine and yet not fully controlled, many of them had become covert susceptible groups. At a time of frequent communication between countries, they are likely to develop sudden outbreaks of infection or sporadic infections. The 2002 outbreak in Taiwan was a case in point. Again, two cases though had had two doses of vaccines (MV at the age of nine months, and MMR at 15 months), had developed typical measles and their IgM antibodies were positive. They could have been cases of primary vaccine failure[8]. In three cases, IgM antibodies did not show, and IgG was positive. Chances are under circumstances where cases are becoming fewer, there is less chance of nature boost, antibodies may possibly decline[9]. Reports have indicated

that antibody titers are lower in vaccination than natural infection[10,11]. Whether measles vaccine will, now that cases are sharply declining, produce life-long immunity should be seriously considered before measles is completely eradicated.

At a time when the world is close to the elimination of measles (interruption of internal transmission of measles), virological and molecular biological monitoring becomes more important. As the number of cases declines, young doctors, for the lack of confrontation with measles, may have difficulties in the diagnosis of cases. By the guidelines of the measles eradication by phase[12,13], each reported case should not only be collected specimen and laboratory-tested for confirmation, his/her specimen in the early stage of infection should also be collected for virus isolation molecular-biologically. Only when the chain of transmission of each case is understood, interruption can be made. Like the eradication of polio, it is only through the global participation and monitoring that the eradication of measles can be possible.

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Prepared by：Cheng WY, Wang SK, Yang CY, Chen HY

Virological Laboratory, Laboratory Testing and Research Division, CDC/DOH

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Table 1. Data of Confirmed Measles Cases, 2002

No	Age (Yr/Mo)	Date of Rash	Collection Date	MV (Immunization Date)	MMR (Immunization Date)	Abroad (Which)
06/02	0/10	91/02/22	91/02/25	Y(91/02/02)	N	N
10/02	1/0	91/02/27	91/03/04	N	N	Y(Philippines)
18/02	9/10	91/04/01	91/04/04	Y(82/03/08)	Y(82/09/27)	N
23/02	29/4	91/04/15	91/04/19	N	Y	N
27/02	0/7	91/06/18	91/06/23	N	N	Y(China-Hunan)
31/02	0/10	91/06/25	91/07/04	N	N	Y(China)
37/02	0/6	91/08/09	91/08/12	N	N	Y(China-Guangdong)
37C2/02	25/2	91/07/27	91/08/14	Unknown	Unknown	Y(China-Guangdong)
38/02	18/7	91/08/04	91/08/14	N	N	N
39/02	27/4	91/08/24	91/08/26	Y	N	N
40/02	19/11	91/08/21	91/08/26	Unknown	Unknown	N
42/02	0/7	91/08/29	91/09/06	N	N	N
44/02	26/1	91/09/08	91/09/13	Y	Y	N
47/02	14/10	91/09/17	91/09/19	Y	Y	N
47C5/02	14/11	91/09/19	91/09/19	Y(77/08/02)	Y(78/01/24)	N
49/02	26/5	91/09/09	91/09/23	Unknown	Y	Y(China)
50/02	9/11	91/09/19	91/09/25	N	N	N
50D18/02	9/1	91/09/29	91/09/27	Y	Y	N
50D26/02	9/7	91/10/02	91/09/27	Y	Y	N
50D35/02	13/2	91/09/30	91/09/30	Y	Y	N
50D37/02	9/11	91/09/30	91/09/30	Y	Y	N
51/02	0/1	91/09/25	91/09/26	N	N	N
51C2/02	26/11	91/09/17	91/10/11	Unknown	Unknown	N
53/02	13/3	91/10/04	91/10/05	Y	Y	N
53D10/02	13/11	91/10/19	91/10/07	Y	Y	N
53D22/02	13/10	91/09/24	91/10/07	Y(78/11/02)	Unknown	N
54/02	29/0	91/10/01	91/10/05	Unknown	Unknown	N

Table 1. Data of Confirmed Measles Cases, 2002（continue）

No	Serological Finding	Remarks	Kind of Specimen [a]	Genome Sequencing and Nomenclature
06/02	IgM(+)	Sporadic	Serum	
10/02	IgM(+)	Sporadic	[Serum]	MVs/ Tai-Zhong.TWN/10.02
18/02	IgM(+)	Sporadic	[Serum], whole blood	MVs/Taipei.TWN/14.02
23/02	IgM(+)	Sporadic	[Serum]	MVs/Kao-Shung.TWN/16.02
27/02	IgM(+)	Sporadic	Serum, throat swab, [urine]	MVs/Taipei.TWN/26.02
31/02	IgM(+)	Sporadic	Serum,[throat swab],urine	MVs/Taipei.TWN/27.02
37/02	IgM(+)	Chain 1	Serum, throat swab,[urine]	MVs/Ping-Tung.TWN/33.02
37C2/02	IgM(+)	Chain 1	Serum	
38/02	IgM(+)	Sporadic	Serum	
39/02	IgM(+)	Sporadic	Serum	
40/02	IgM(+)	Sporadic	Serum	
42/02	IgM(+)	Sporadic	Serum,[throat swab],urine	MVs/ Tai-Zhong.TWN/36.02
44/02	IgM(+)	Sporadic	whole blood, throat swab, urine	
47/02	IgM(+)	Chain 2	Whole blood, Serum,[urine]	MVs/ Tai-Zhong.TWN/38.02
47C5/02	IgM(+)	Chain 2	Serum	
49/02	IgM(+)	Sporadic	Serum	
50/02	IgM(+)	Chain 3	serum, urine	
50D18/02	IgM(+)	Chain 3	Serum	
50D26/02	IgM(+)	Chain 3	Serum	
50D35/02	IgM(+)	Chain 3	Serum,[urine]	MVi/Tai-Zhong.TWN/40.02/1
50D37/02	IgM(+)	Chain 3	Serum,[urine]	MVi/Tai-Zhong.TWN/40.02/2
51/02	IgM(+)	Chain 4	[Serum]	MVs/ Hsin-Chu.TWN/39.02
51C2/02	IgM(+)	Chain 4	Serum	
53/02	IgM(+)	Chain 5	[whole blood], throat swab, urine	MVi/Tai-Zhong.TWN/40.02/3
53D10/02	IgM(+)	Chain 5	Serum	
53D22/02	IgM(+)	Chain 5	Serum	
54/02	IgM(+)	Sporadic	Whole blood、[Serum]	MVs/ Hsin-Chu.TWN/40.02/4

a：「 」Specimens in [ ] refer to sources of specimens in which either viruses were isolated or they were genome sequenced

Table 2. Reference Strains for Phylogenetic Analysis of Wild-type Measles Viruses, 2002

Genotype	Status[a]	Reference strain(MVi)[b]	H gene Accession	N gene Accession
A	Active	Edmonston-wt.USA/54	U03669	U01987
B1	Active	Younde.CAE/12.83"Y-14"	AF079552	U01998
B2	Active	Libreville.GAB/84"R-96"	AF079551	U01994
B3	Active	New York.USA/94 Ibadan.Nie/97/1	L46752 AJ239133	L46753 AJ232203
C1	Active	Tokyo.JPN/84/K[c]	AY047365	AY043459
C2	Active	Maryland.USA/77"JM" Erlangen.DEU/90"WTF"	M81898 Z80808	M89921 X84872
D1	Inactive	Bristol.UNK/74(MVP)	Z80805	D01005
D2	Active	Johannesburg.SOA/88/1	AF085198	U64582
D3	Active	Illinios.USA/89/1"Chicago-1"	M81895	U01977
D4	Active	Montreal.CAN/89	AF079554	U01976
D5	Active	Palau.BLA/93 Bangkok.THA/93/1	L46757 AF009575	L46758 AF079555
D6	Active	New Jersey.USA/94/1	L46749	L46750
D7	Active	Victoria.AUS/16.85 Illinois.USA/50.99	AF247202 AY043461	AF243450 AY037020
D8	Active	Manchester.UNK/30.94	U29285	AF280803
E	Inactive	Goettingen.DEU/71"Braxator"	Z80797	X84879
F	Inactive	MVs/Madrid.SPA/94 SSPE	Z80830	X84865
G1	Inactive	Berkeley.USA/83	AF079553	U01974
G2	Active	Amsterdam.NET/49.97	AF171231	AF171232
g3[d]	Active	MVs/Victoria.AUS/24.99	AF353621	AF353622
H1	Active	Hunan.CHN/93.7	AF045201	AF045212
H2	Active	Beijing.CHN/94/1	AF045203	AF045217

Notes:
(a) Active refers to strains isolated in the last 15 years.
(b) WHO nomenclature; names quoted by other reports are shown in ( ).
(c) Associated strain, MVi/Tokyo.JPN/84/K is used to replace the previous genotype C1 reference strain.
(d) Hypothetical new genotype, waiting for isolation of reference strains.

Table 3. Global Distribution of Known Wild-Type Measles Viruses

Genotype	Areas of local cases, outbreak, or sources of imported cases，1995-2001
B1	Cameroon, by strains isolated in early 1980's
B2	Gabon, by strains isolated in early 1980's
B3	Congo, Democratic Republic of Congo, Gambia, Ghana, Kenya, Nigeria, Sudan
C2	Czech Republic, Denmark, Germany, Luxembourg, Morocco, Spain
D2	Ireland, outbreaks in 2000, South Africa, Zambia
D3	Japan, the Philippines[a]
D4	Ethiopia, India, Iran, Kenya, Namibia, Pakistan, Russian Federation, South Africa, Zimbabwe
D5	Japan, Namibia, Thailand
D6	Argentina, Brazil, Bolivia, Dominican Republic, Germany, Italy, Luxembourg, Poland, Russian Federation, Spain, Turkey
D7	Germany, Spain
D8	Ethiopia, India, Nepal
G2	Indonesia, Malaysia
g3[b]	East Timor
H1	China, Republic of Korea
H2	Viet Nam

Notes:

a, could have been imported from the Philippines.

b, hypothetic new genotype, waiting for isolation of reference strains.

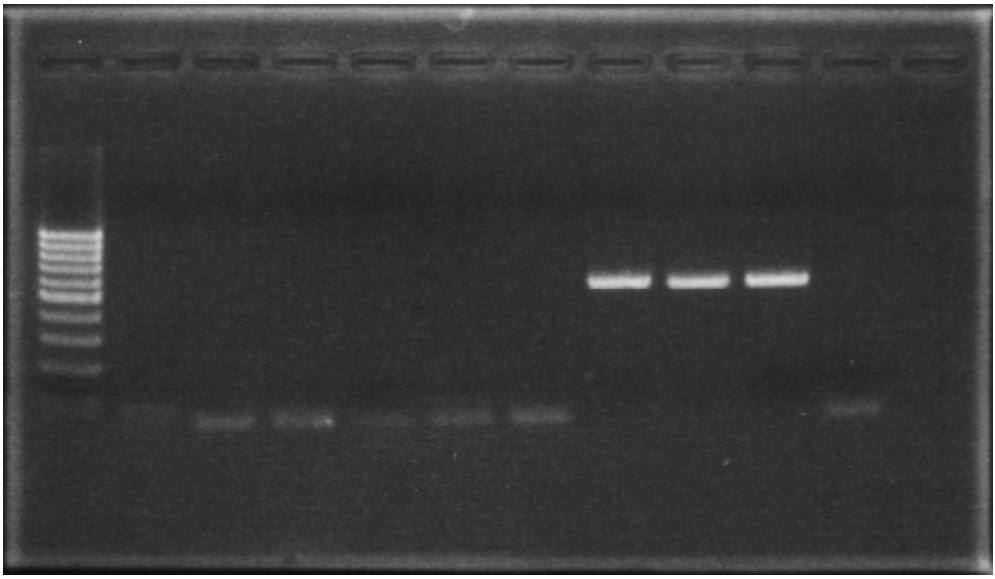
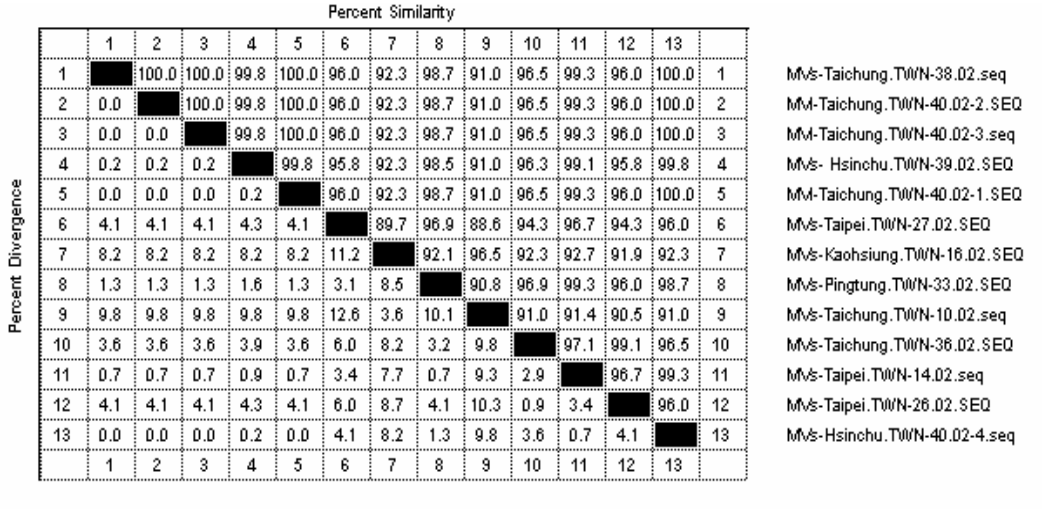


Figure 1. The 589 bps section collected after augmentation with nest-PCR using MV60 and MV63 as primers. The figure on the far-left end is the 100 bp ladder marker.



	1	2	3	4	5	6	7	8	9	10	11	12	13	
1		100.0	100.0	99.8	100.0	96.0	92.3	98.7	91.0	96.5	99.3	96.0	100.0	1
2	0.0		100.0	99.8	100.0	96.0	92.3	98.7	91.0	96.5	99.3	96.0	100.0	2
3	0.0	0.0		99.8	100.0	96.0	92.3	98.7	91.0	96.5	99.3	96.0	100.0	3
4	0.2	0.2	0.2		99.8	95.8	92.3	98.5	91.0	96.3	99.1	95.8	99.8	4
5	0.0	0.0	0.0	0.2		96.0	92.3	98.7	91.0	96.5	99.3	96.0	100.0	5
6	4.1	4.1	4.1	4.3	4.1		89.7	96.9	88.6	94.3	96.7	94.3	96.0	6
7	8.2	8.2	8.2	8.2	8.2	11.2		92.1	96.5	92.3	92.7	91.9	92.3	7
8	1.3	1.3	1.3	1.6	1.3	3.1	8.5		90.8	96.9	99.3	96.0	98.7	8
9	9.8	9.8	9.8	9.8	9.8	12.6	3.6	10.1		91.0	91.4	90.5	91.0	9
10	3.6	3.6	3.6	3.9	3.6	6.0	8.2	3.2	9.8		97.1	99.1	96.5	10
11	0.7	0.7	0.7	0.9	0.7	3.4	7.7	0.7	9.3	2.9		96.7	99.3	11
12	4.1	4.1	4.1	4.3	4.1	6.0	8.7	4.1	10.3	0.9	3.4		96.0	12
13	0.0	0.0	0.0	0.2	0.0	4.1	8.2	1.3	9.8	3.6	0.7	4.1		13
	1	2	3	4	5	6	7	8	9	10	11	12	13	

Figure 2. Sequencing of the 456 bps at the COOH end of the N protein of the wild-type measles strain, 2002. Similarity of nucleic acid (right upper half) and dissimilarity (left lower half) are compared.

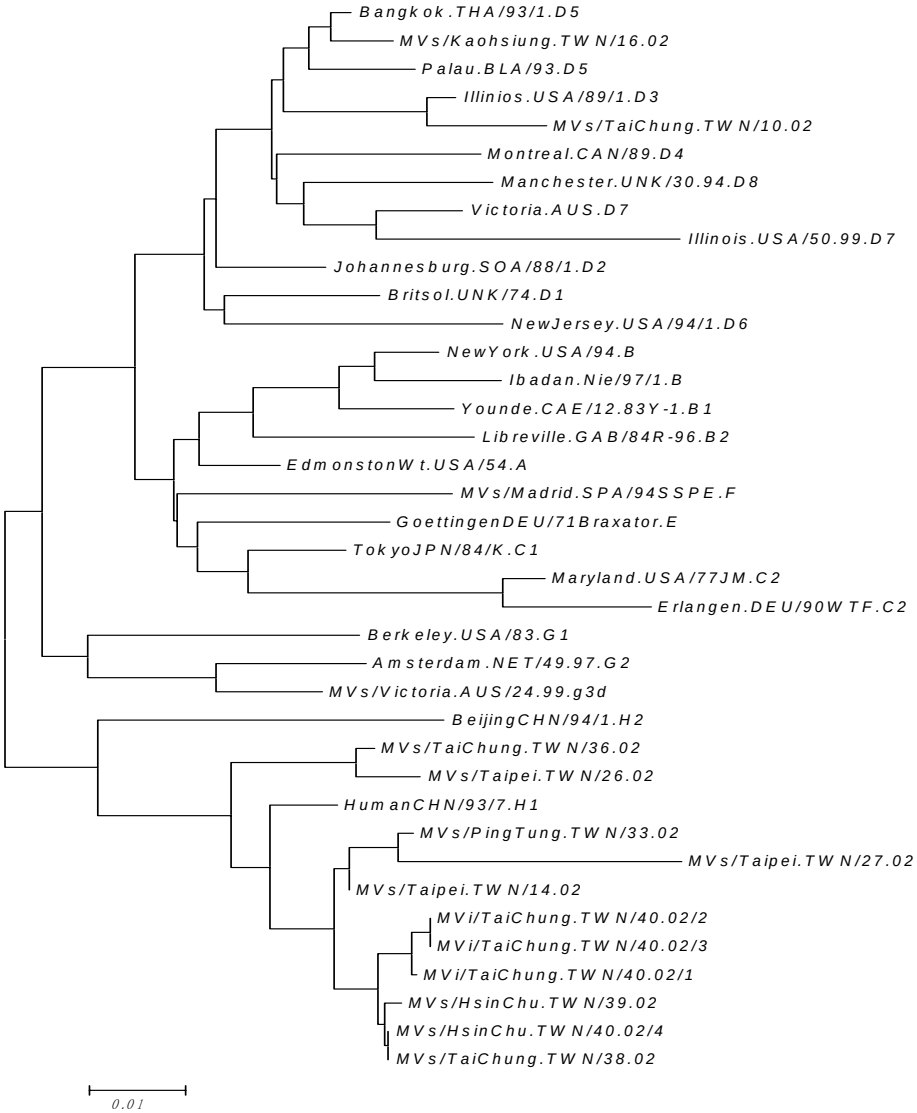


Figure 3. Phylogenetic tree analysis the genome sequences of wild-type measles strains, 2002; Molecular Evolutionary Genetic Analysis (MEGA) version 2.1 was used for analysis with the "neighbor-joining" method, and bootstrapped for 1,000 times.