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Molecular characteristics of mumps viruses isolated in Taiwan from 2006 to 2016

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Abstract

Sixteen mumps virus (MuV) sequences collected in Taiwan between 2006 and 2016 were characterized as genotype F (n = 1), G (n = 7), H (n = 4), J (n = 2), and K (n = 2). Mumps genotype F strain was imported from China in 2008 which was in accordance with the epidemic genotype in China. The Philippines was indicated as export country of three genotype H strains in 2007–2010 and Vietnam as export country of one genotype K strain in 2016 that matched with genotypes described in previous reports. Four strains of genotype G were imported from Japan, Thailand, Malaysia and Myanmar individually indicated that genotype G spreads widely in Asia as well as in the global. In this study, mumps strains of genotype G was first reported in relation to import from Malaysia and Myanmar. Furthermore, Indonesia was referred to export MuV of genotype J in 2007 for the first time. Molecular genotyping benefits the differentiation of circulating mumps viruses and can be used to investigate the transmission pathways. The dynamic genotypes of imported cases revealed the epidemic genotypes in nearby countries.

Keywords: Infectious disease, Genetics, Virology, Epidemiology

1. Introduction

Mumps is a common childhood disease which is transmitted through respiratory secretions, direct contact, or contaminated objects. The classic symptom of mumps

is painfully swollen parotid salivary glands (parotitis) (Latner and Hickman, 2015). Some complications of infection include hearing loss, orchitis, oophoritis, aseptic meningitis may occur (Rubin et al., 2015). However, it also has been estimated that as many as 30% infections in unvaccinated individuals may be asymptomatic (Dittrich et al., 2011).

Mumps is a vaccine preventable disease, the live attenuated mumps vaccine was first licensed in the US in 1967, and since then, it had been widely used as a component of the trivalent measles- mumps –rubella (MMR) vaccine. In Taiwan, measles, mumps and rubella (MMR) vaccine had been included in national immunization program in 1992 for 15 months old infants, and the two- dose MMR program for 12–15 months old infants and first graders (6 years old) was implanted in 2006, the coverage rate of the MMR vaccine has reached 95% since 1996. Mumps outbreak had never been reported in Taiwan since 1992. Mumps was listed as a reportable disease in Taiwan under the clinical criteria of acute, painfully self-limited swelling of single or both salivary glands and lasted for 2 days with no other reasons. Though the reported mumps cases maintained at three to twelve hundreds a year since 2001, but less than 10% of reported cases were laboratory-confirmed except for 2006–2007, over 95% reported cases (about 2000 cases in total) had specimens collected for a research plan to realize the true incidence of mump, under the laboratory diagnostic criteria for mumps case by either positive IgM in serum or positive RT-PCR result in throat swab or urine, only 5 and 11 cases each in 2006 and 2007 fulfilled the criteria of laboratory diagnosis for mumps.

Mumps virus (MuV) belongs to the genus *Rubulavirus* in the family *Paramyxoviridae*, is a non-segmented, single-stranded, negative sense RNA virus that contains 15,384 nucleotides in length. It encodes seven tandemly linked transcription units: the nucleo-(N), V/phosphor-/I (V/P/I), matrix (M), fusion (F), small hydrophobic (SH), hemagglutinin-neuraminidase (HN), and large (L) proteins (Elango et al., 1988).The characterization of MuV diversity is based from studying nt sequences of its most variable gene, SH (Jin et al., 2005). This sequence includes the non-coding regions flanking the coding sequence for the SH protein. Based on the nucleotides sequences of the small hydrophobic (SH) gene and the hamagglutinin-neuraminidase (HN) gene, 12 MuV genotypes A, B, C, D, F, G, H, I, J, K, L, and N have been identified so far (WHO, 2012).

Genotyping of the virus plays an important role in MuV surveillance. The global distribution of MuV genotypes is poorly described, till 2013, genotype data were available at 44/194 countries globally (Jin et al., 2015). To address the geographical and chronological change of MuV genotypes, here we report 5 genotypes (F, G, H, J, K) collected in Taiwan between 2006 and 2016 were

imported from 8 countries (China, Indonesia, Japan, Malaysia, Myanmar, the Philippines, Thailand and Vietnam), by the epidemiologic investigation.

2. Materials and methods

2.1. Clinical samples

Clinical samples that yielded a complete MuV SH gene, HN gene and complete or partial F gene sequence from patients diagnosed between 2006 and 2016 were used in this study. Analyzed samples included 15 throat swabs and 1 urine specimens. Epidemiological data about age, nationality of patient, travel history, and date of specimens collected was retrieved from the National Notifiable Disease Report System in Taiwan to provide background for analysis of clustering based on molecular data.

2.2. RNA extraction and nested RT-PCR amplification

Viral RNA was extracted directly from clinical specimens by using QIA-amp mini viral RNA extraction kit (Qiagen, Hilden, Germany). To amplify the SH gene, one-step reverse-transcription PCR(RT-PCR) was performed by using one-step RT-PCR kit (Qiagen, Hilden, Germany) with primers listed in [Table 1](#), and the RT-PCR condition was as follow: reverse transcription step at 50 °C for 30 min, followed by initial PCR activation step at 95 °C for 15 min; 35 cycles of 30 sec at 95 °C, 30 sec at 51 °C, 1 min at 72 °C, and a final extension step of 5 min at 72 °C. A nested PCR that amplified 3 µl of first PCR product was performed using fast PCR master mix (Takara, Kusatsu, Shiga, Japan), the PCR condition was as follow: initial PCR activation step at 95 °C for 1 min, followed by 30 cycles of 5 sec at 94 °C, 5 sec at 55 °C, 10 sec at 72 °C. The full-length of F (1617 nts) and HN (1749 nts) genes were amplified with 3 sets of nested RT-PCR reaction each ([Table 1](#)), the reaction condition for RT-PCR and nested PCR were the same as used in SH gene except for primer 4350F and 5310R for the F gene, the annealing temperature was set to 48 °C.

2.3. Sequencing and phylogenetic analysis

Sequencing reactions were performed by using the ABI BigDye Terminator V3.1 cycle sequencing kit (Life technologies) according to the manufacturer's instruction. The inner primer pairs used in nested RT-PCR reactions were used as sequencing primers for SH, F, and HN gene. All sequences were submitted to the GenBank database and were available under accession number MF280977-MF280992 for F gene, MF280993-MF281008 for HN gene, and MF 281009-MF281024 for SH gene.

Table 1. Primers used for amplifying and sequencing of the SH, F and HN genes.

Name	Sequence(5'-3')	location	Note
SH-F1F	gtagcagccttagtttgagcat	6007-6029	SH outer primer (Jin et al., 1999; Lee et al., 2003)
SH-SH2R	gctcaagccttgatcattga	6789-6808	
SH-SH1F	agttagtgcgatgatctcat	6133-6152	SH inner primer(Jin et al., 1999; Lee et al., 2003)
SH-HN3R	tgaagagttttgagggctccat	6614-6635	
F-4350F	tggagtccRttYaggaagtc	4350-4369	F outer primer set 1
F-5310R	cattaRaccggcRcttagtat	5290-5310	
F-4371F	gcctaaatgagYaacctRtg	4371-4390	F inner primer set 1
F-5170R	gctgRaacactgtgtYaRtt	5150-5170	
F-4928F	gtKcaagaacagacRaatgc	4927-4947	F outer primer set 2
F-5680R	tgcaacaattgtYccatcca	5660-5680	
F-4848F	aMggtttgcYggYattgcYat	4848-4868	F inner primer set 2
F-5647R	gcctcatataRctYcctgcta	5627-5647	
F-5382F	acacaatcaaaYgcattRgt	5382-5402	F outer primer set 3
F-6275R	gScggcatagtcDacggca	6255-6275	
F-5406F	gacttctacKcaattcRag	5406-5426	F inner primer set 3
F-6254R	gtcacgagacgttacgaccc	6234-6253	
HN-6531F	aaaScaRgccMgaacaaRct	6531-6550	HN outer primer set 1
HN-7336R	ccgattYaggccatcactga	7317-7336	
HN-6580F	ctgctateWcaaYtgtgctc	6580-6599	HN inner primer set 1
HN-7303R	aaRgttttgaacatRggat	7284-7302	
HN-7253F	atggggattctcgttcaRac	7253-7272	HN outer primer set 2
HN-7980R	tgaaccaggacgagtgaatg	7980-7999	
HN-7260F	ttctcgttcaRacBgcKtca	7260-7279	HN inner primer set 2
HN-7961R	gaRtatataggtatccagga	7961-7980	
HN-7900F	tgaactcctctaYgagatat	7900-7919	HN outer primer set 3
HN-8479R	taaagtacttcaggtarGa	8460-8479	
HN-7910F	taYgagatatcattcacMtt	7910-7928	HN inner primer set 3
HN-8438R	ttctgcctgtttttctta	8419-8438	

The related location of each primer was compared with whole genome AF338106.

Representative F gene sequences of mumps genotype D, F, J, K and L were according to Lin et al. (Jin et al., 2015), and the other genotype of mumps F, SH and HN gene sequences were referred to WHO reference strains (WHO, 2012). The phylogenetic trees were drawn on the basis of the SH and HN gene individually using the neighbor-joining method in MEGA software (version 6.0) with bootstrapping (500 replicates).

3. Results

3.1. Epidemiological data

The 16 specimens were collected from mumps patients with age distribution in 3–50 years old, over 80% (13 cases) were foreigners, and over 60% (10 cases) were imported cases from the other countries. The strain name, GenBank accession number of F, SH and HN gene of each sequences were shown in [Table 2](#). The identities of these non-native residents including travelers, foreign workers, and foreign spouses, and according to the travel history 2 to 3 weeks ago before symptom onset to indicate the imported country of each case, 10 imported cases were identified from eight countries including China, Malaysia, Myanmar, Japan, Indonesia, Thailand, the Philippines, and Vietnam. Through the genotype and export country of each imported case, implied that genotype F MuVs circulated in China in 2008; genotype G MuVs circulated in Malaysia in 2006, in Myanmar in 2007, in Thailand in 2009, and in Japan in 2015; genotype H MuVs circulated in Philippines in 2007–2010; genotype J MuVs circulated in Indonesia in 2007; and genotype K MuVs circulated in Vietnam in 2016.

3.2. Phylogenetic analysis

Complete SH gene and HN gene sequences were obtained from 16 clinical specimens collected from 2006 to 2016. Phylogenetic analysis of SH ([Fig. 1](#)) and HN ([Fig. 2](#)) gene sequences with reference strains of 12 mumps genotypes showed concordant results of genotype designation. To investigate the transmission routes of 6 source unidentified MuVs ([Table 2](#)), the SH gene sequences of four genotype G, H, J, and K identified in Taiwan were compared further with sequences ([Supplementary Table 1](#)) from the other countries to find the geographical and chronological relationship.

3.2.1. Sub tree of genotype G

Compared the SH gene sequences of 7 genotype G MuVs from Taiwan between 2006 and 2016 with that from the United Kingdom (UK) in 1996–2005 ([Cui et al., 2009](#); [Jin et al., 2004](#)), the Netherland in 2004–2015 ([Gouma et al., 2016](#)), Japan ([Inou et al., 2004](#); [Momoki, 2013](#)), the United State of America (USA) ([Rota et al., 2009](#)), Canada ([Watson-Creed et al., 2006](#)), the other countries that described in the global distribution of MuV genotype ([Jin et al., 2015](#)) and the recently circulated MuV sequences that deposited in GenBank, could found two clusters of genotype G ([Fig. 3](#)), the major MuVs from cluster 1 were isolated in countries from Europe or America including the UK, the Netherland, Canada and the USA, relative to cluster 1, MuVs of cluster 2 were isolated in countries from Asia, the majority were from Japan or imported from Japan. For example, sequence from mumps outbreak occurred at the University of Virginia in 2006(FJ959106) was

Table 2. Characteristics of 16 mumps virus strains in Taiwan, 2006-2016.

Age (yy/mm)	Patient's Nationality ^a	Export Country ^a	Strain Name	GenBank accession Number(F/SH/HN)
11/01	TWN	CHN	MuVs/Kaohsiung.TWN/06.08[F]	MF280981/MF281015/MF281008
10/03	TWN	MYS	MuVs/Taipei.TWN/51.06[G]	MF280991/MF281011/MF281007
34/08	MMR	MMR	MuVs/Taipei.TWN/46.07[G]	MF/280980/MF281013/MF281000
35/9	USA	unidentified	MuVs/Taipei.TWN/50.07[G]	MF/280992/MF281014/MF280992
3/10	JPN	unidentified	MuVs/Kaohsiung.TWN/05.09[G]	MF/280982/MF281016/MF281002
33/10	THA	THA	MuVs/Taipei.TWN/19.09[G]	MF/280988/MF281017/MF281003
36/05	TWN	JPN	MuVs/Kaohsiung.TWN/11.15[G]	MF/280986/MF281022/MF281005
49/03	KOR	unidentified	MuVs/Taipei.TWN/40.15[G]	MF280987/MF281023/MF280995
26/09	PHL	PHL	MuVs/Kaohsiung.TWN/42.07[H]	MF/280979/MF281012/MF/280999
13/01	IDN	unidentified	MuVs/Taipei.TWN/26.09[H]	MF/280984/MF281018/MF281006
25/10	PHL	PHL	MuVs/Taipei.TWN/48.09[H]	MF280985/MF281019/MF281004
26/02	PHL	PHL	MuVs/Tainan.TWN/18.10[H]	MF280989/MF281021/MF/280994
23/10	IDN	unidentified	MuVs/Taichung.TWN/27.06[J]	MF/280977/MF281010/MF/280997
38/09	IDN	IDN	MuVs/Taichung.TWN/39.07[J]	MF/280978/MF281009/MF/280998
50/7	USA	unidentified	MuVs/Taoyuan.TWN/10.10[K]	MF/280990/MF281020/MF280996
30/6	VNM	VNM	MuVs/Taichung.TWN/48.16[K]	MF280983/MF/281024/MF280993

^a Country abbreviation is according to ISO3 country codes: CHN: China, IDN: Indonesia, JPN: Japan, KOR: Republic of Korea, MMR: Myanmar, MYS: Malaysia, PHL: Philippine, THA: Thailand, USA: United States of America, VNM: Viet Nam. TWN refer Taiwan.

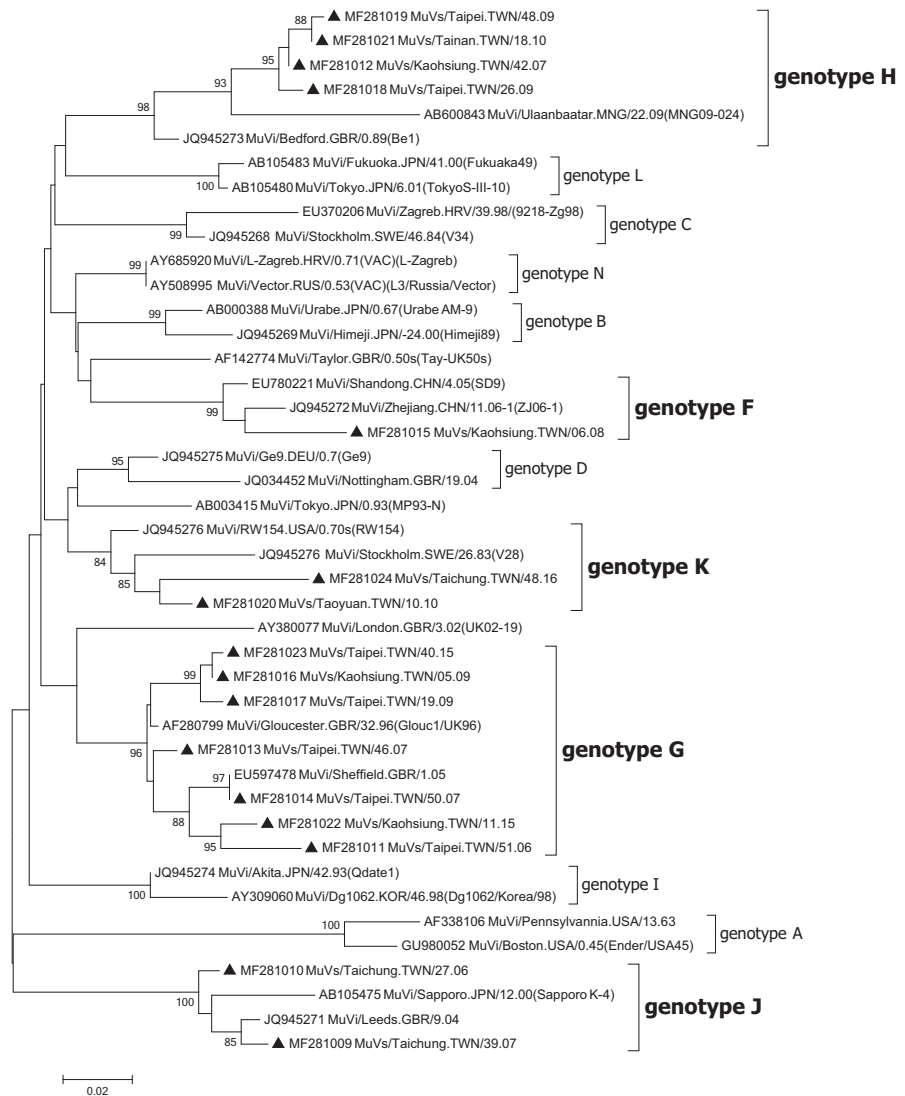


Fig. 1. Phylogenetic analysis of 16 mumps strains obtained from 2006 to 2016 based on 316 nucleotides of the SH gene with reference strain of 12 genotypes (A, B, C, D, F, G, H, I, J, K, L and N). The sequences for SH gene of 16 Taiwan's isolates were deposited into GenBank under accession numbers MF281009-MF281024 and indicated by (▲). Bootstrap values higher than 70 in the branches are shown. The un-rooted neighbor-joining consensus tree was generated by bootstrap analysis of 500 replicates using the MEGA 6.0 software (www.megasoftware.net). Scale bar indicates nucleotide substitutions per site.

similar to an imported case from Japan (FJ959107) in New Hampshire in 2005 (Rota et al., 2009). MuVs of cluster 1 could be further divided into two sub-clusters, in an earlier report be identified as G2(EU597476) and G5(EU597478) (Cui et al., 2009), moreover, the MuVs from outbreak in the Netherland in 2004-2015(KJ125045,KJ125051) (Gouma et al., 2016), in Canada in 2005-2006 (DQ664492,DQ664493) (Watson-Creed et al., 2006) and MuVs from the USA in 2010–2017 together formed sub cluster G5 05–17, on the other hand, MuVs

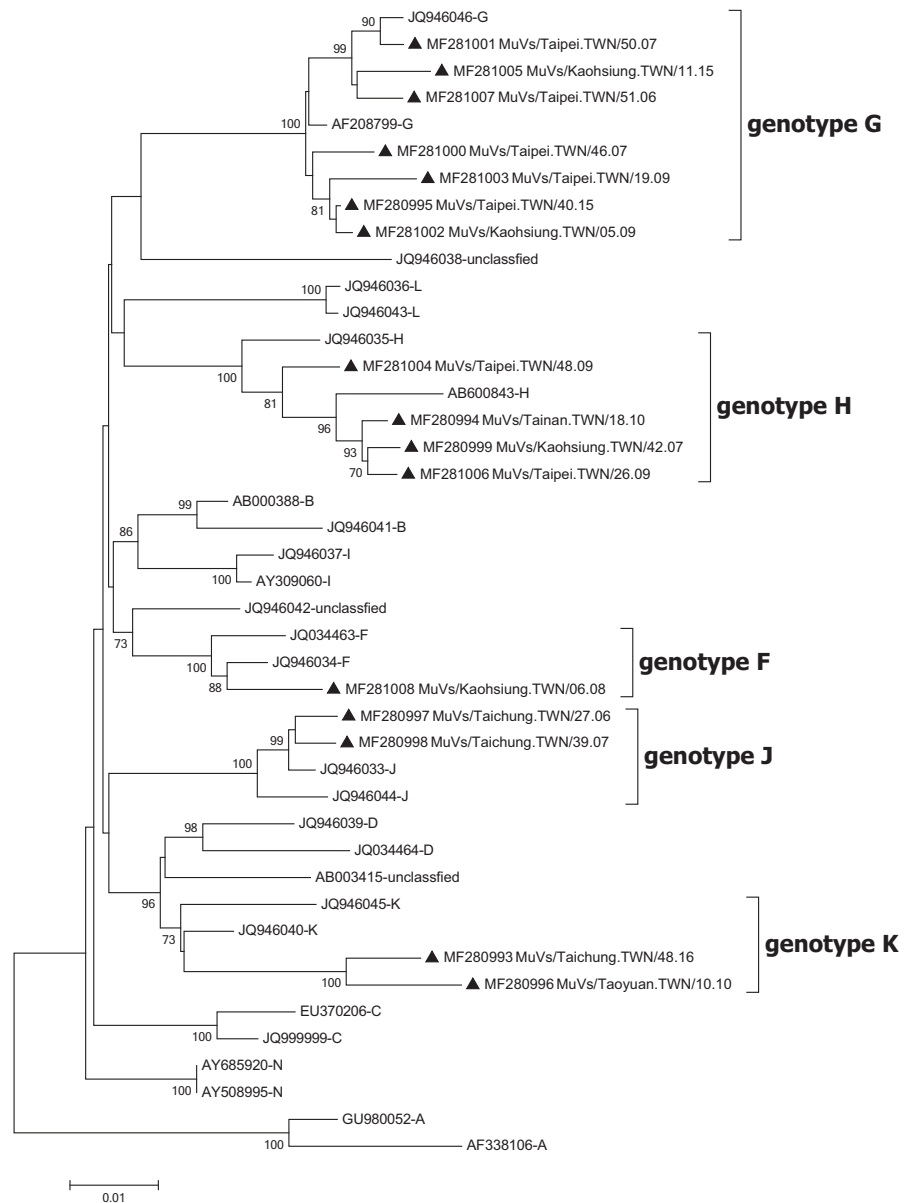


Fig. 2. Phylogenetic analysis of 16 mumps strains obtained from 2006 to 2016 based on 1749 nucleotides of the HN gene with reference strain of 12 genotypes (A, B, C, D, F, G, H, I, J, K, L and N). The sequences for HN gene of 16 Taiwan's isolates were deposited into GenBank under accession numbers MF280995-MF281008 and indicated by (▲). Bootstrap values higher than 70 in the branches are shown. The un-rooted neighbor-joining consensus tree was generated by bootstrap analysis of 500 replicates using the MEGA 6.0 software (www.megasoftware.net). Scale bar indicates nucleotide substitutions per site.

isolated at Yokohama city in Japan in 2010 (AB699709,AB699710) (Momoki, 2013), at Guam in 2010 (JX455900,JX455905), at India (KF783114,KY826490) and two Taiwan's isolates MuVs/Kaohsiung.TWN/11.15[G] and MuVs/Taipei.TWN/51.06[G] were imported from Japan and Malaysia individually, together

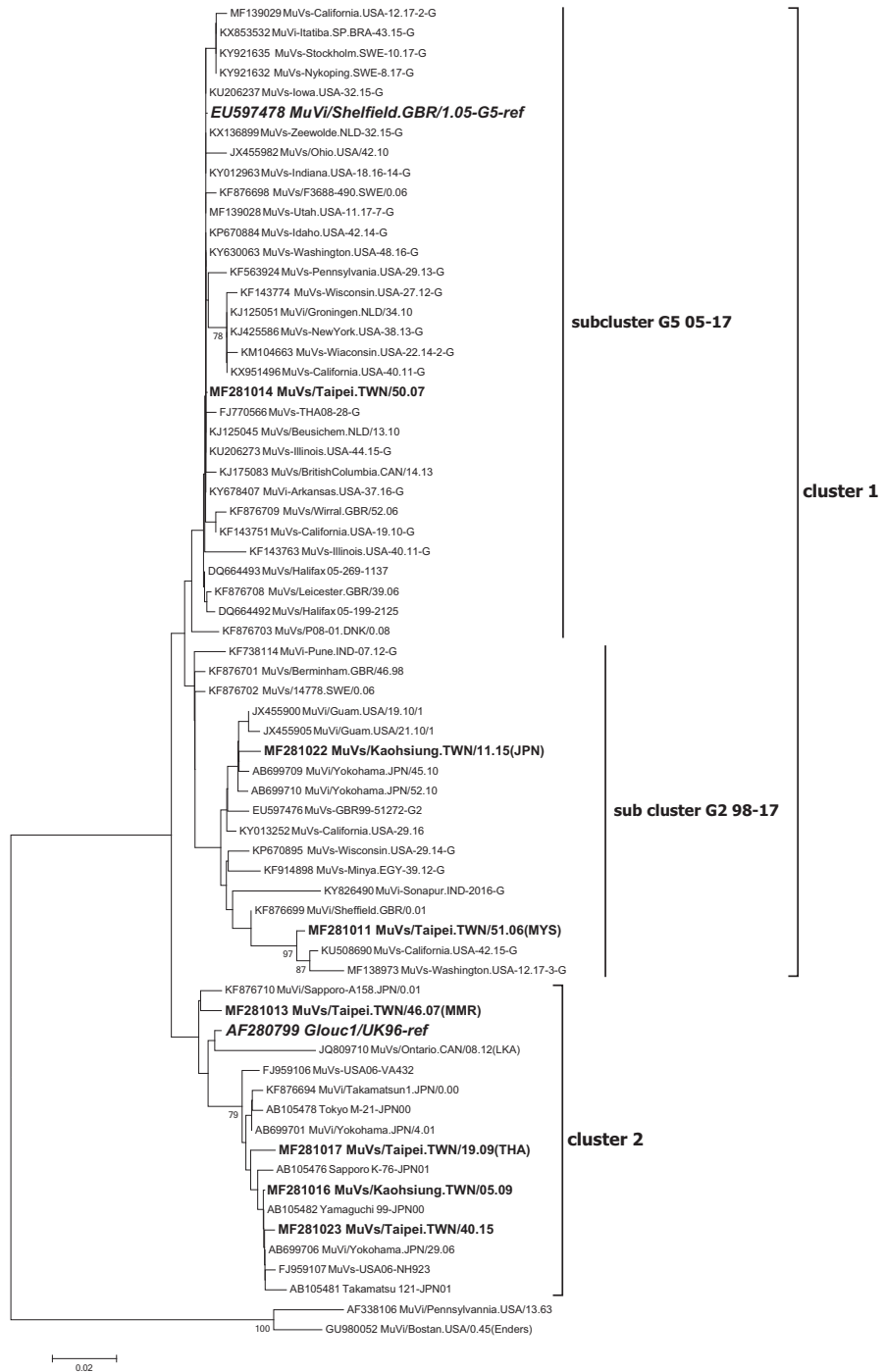


Fig. 3. Phylogenetic analysis of mumps viruses based on SH gene of Taiwan’s isolates and strains isolated in other countries for genotype G, strains for reference genotype A were included as outlier of each genotype. Strain names from reference genotype are shown as bold, italic, and Taiwan’s isolates are shown as bold. Bootstrap values higher than 70 in the branches are shown. The accession number and reference journal for each sequence that used to picture the sub-tree for genotype G was listed in Supplementary Table 2, the letters in parenthesis after some strain name indicated export country and shown as country abbreviation according to ISO3 code.

formed sub cluster G2 98–17. Another imported strain (MuVs/Taipei.TWN/19.09 [G]) from Thailand was distributed in cluster 2, different from another Thailand's isolate in 2008 (FJ770566), which was located in cluster 1, sub-cluster G5 05–17 (Jin et al., 2015). Three MuVs could not be linked to importation through epidemic investigation, among those, MuVs/Taipei.TWN/50.07[G] was isolated from an American and distributed to sub-cluster G5 05–17, the epidemic linked to countries of Europe or America was speculated. The other two MuVs of MuVs/Kaohsiung. TWN/05.09[G] and MuVs/Taipei.TWN/40.15[G] were isolated from patients of Japanese and South Korean individually, the highly sequences similarity of these two strains with Japan's isolate in 2000 (AB105482) and 2006 (AB699706) (Inou et al., 2004; Momoki, 2013) indicated the continuing circulation of viruses in this lineage. MuVs/Taipei.TWN/46.07[G] was an imported MuV from Myanmar, showed deviation from most strains from Japan in cluster 2.

3.2.2. Sub tree of genotype H

Four genotype H strains were isolated in Taiwan, except one strain MuVs/Taipei. TWN/26.09[H] with unidentified source, the other three strains MuVs/Kaohsiung. TWN/42.07[H], MuVs/Taipei.TWN/48.09[H], MuVs/Tainan.TWN/18.10[H] were isolated from Filipinos came to Taiwan from the Philippines recently. Compared MuV sequences with that from the other countries, two clusters were identified (Fig. 4). Cluster 1 composed of MuVs circulated in countries from Europe (Belarus, Israel, Italy, Spain, Sweden, Switzerland, Turkey and UK) (Akcali et al., 2009; Atrasheuskaya et al., 2007; Hindiyeh et al., 2009; Montes et al., 2002; Utz et al., 2004) and America (the USA, Dominican Republic-JQ034432,), while cluster 2 was composed of MuVs from Asia (Mongolia, South Korea, the Philippine, and Japan).The MuVs of genotype H that cause outbreaks in South Korea in 1999 (sub cluster KOR 99) (Lee et al., 2003) could be differentiated from MuVs circulated in 2008–2012 (sub cluster KOR 08–12) and the other outbreak strains of Mongolia in 2009 (sub cluster MNG 09) (Kidokoro et al., 2011). The imported MuVs from the Philippines as indicated in Taiwan and Canada (JQ783112,JQ809709), MuVs from Kobe City, Japan (sub cluster JPN 10) (Akiyoshi and Suga, 2014), and MuVs circulated in South Korea in 2008–2012 (sub cluster KOR 08–12) composed of sub cluster Asia 04–12.While strains MuVs/BritishColumbia.CAN/01.12(JQ783116) had been indicated as imported from South Africa, similar sequences deposited in GenBank including MuVi/Morelex. MEX/12.13, MuVs/Stockholm.SWE/36.14, MuVs/Minnesota.USA/16.14, MuVs/California.USA/19.14, MuVs/California.USA/36.15 formed sub cluster America 12–15 implied the continuing transmission. Another strain (MuVs/Calgary.CAN/30.07. JN687468) exported from Sudan, a country of WHO Eastern Mediterranean Region, seemed to distribute in a discrete branch other than cluster 1, 2 or sub cluster America 12-15

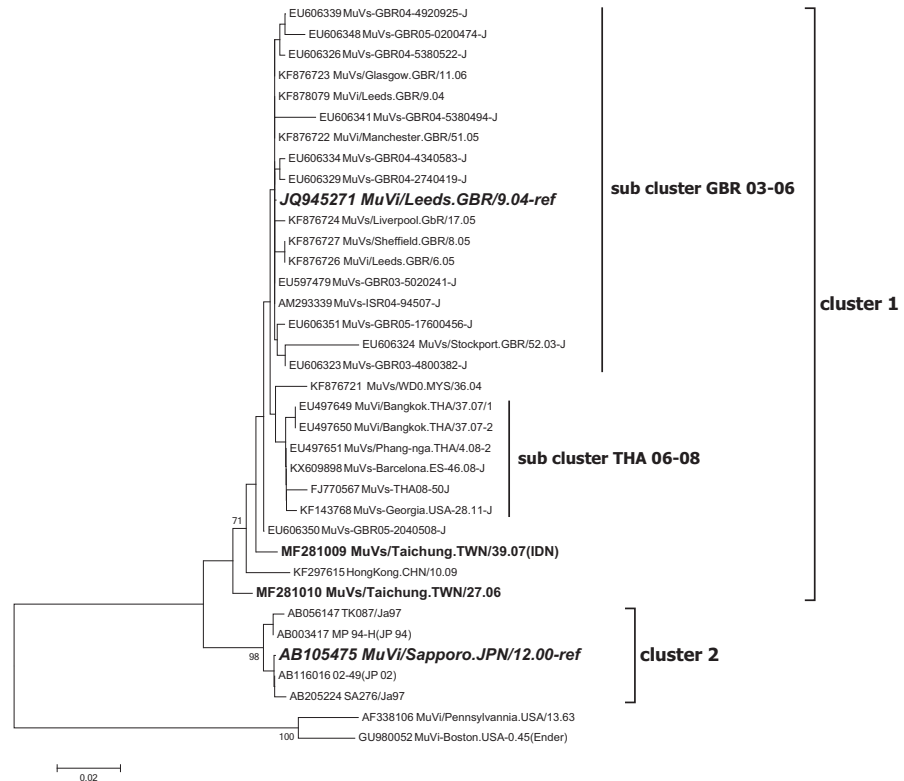


Fig. 5. Phylogenetic analysis of mumps viruses based on SH gene of Taiwan's isolates and strains isolated in other countries for genotype J, strains for reference genotype A strains were included as outlier of each genotype. Strain names from reference genotype are shown as bold, italic, and Taiwan's isolates are shown as bold. Bootstrap values higher than 70 in the branches are shown. The accession number and reference journal for each sequence that used to picture the sub-tree for genotype J was listed in Supplementary Table 2, the letters in parenthesis after some strain name indicated export country and shown as country abbreviation according to ISO3 code.

divided into sub cluster GBR 03–06 that correlated with MuVs identified at the United Kingdom in 2003–2006 (Cui et al., 2009; Jin et al., 2015) and sub cluster THA 06–08 including MuVs isolated in Thailand (EU497649–EU497651, FJ770567), a Malaysian strain (KF 876721) isolated in 2004 clustered closer to MuVs in sub cluster THA 06–08 than strains that detected in sub cluster GBR 03–06, another four strains, including one identified at United Kingdom (EU606350) in 2005, one from Hong-Kong (KF297615) in 2009, and two Taiwan's isolates of MuVs/Taichung.TWN/27.06[J] with unidentified source and MuVs/Taichung.TWN/39.07[J], which was imported from Indonesia, did not formed a cluster with each other; cluster 2 was composed of MuVs from Japan in 1994–2002 (Jin et al., 2015; Kashiwagi et al., 1999; Uchida et al., 2001).

3.2.4. Sub tree of genotype K

Two reference genotype K MuVs, MuVi/RW154.USA/0.70s and MuVi/Stockholm.SWE/26.83, were isolated in 1970s–1980s, mumps epidemic strains from

Spain in 1988–1990 (Cilla et al., 2014) formed sub cluster ESP 87–90 and one reference strain MuVi/Stockholm.SWE/26.83 was included. The sequences of MuVs from outbreak in Brazil between 2006 and 2007 (Santos et al., 2008) were identical to sporadic strains found in the USA (MuV/California.USA/50.07/1, JX287386), Canada (MuVs/BritishColumn.CAN/30.07,KJ1750852), together with the other Brazilian MuVs circulated in 2012–2015, formed sub cluster America 06–15 that deviated from sub cluster ESP 87–90 or MuVs from Vietnam (Fig. 6). MuVs/Taoyuan.TWN/10.10[K] was isolated from an American, who travelled to Thailand during March 1 to March 9, 2010, and came to Taiwan at March 10,

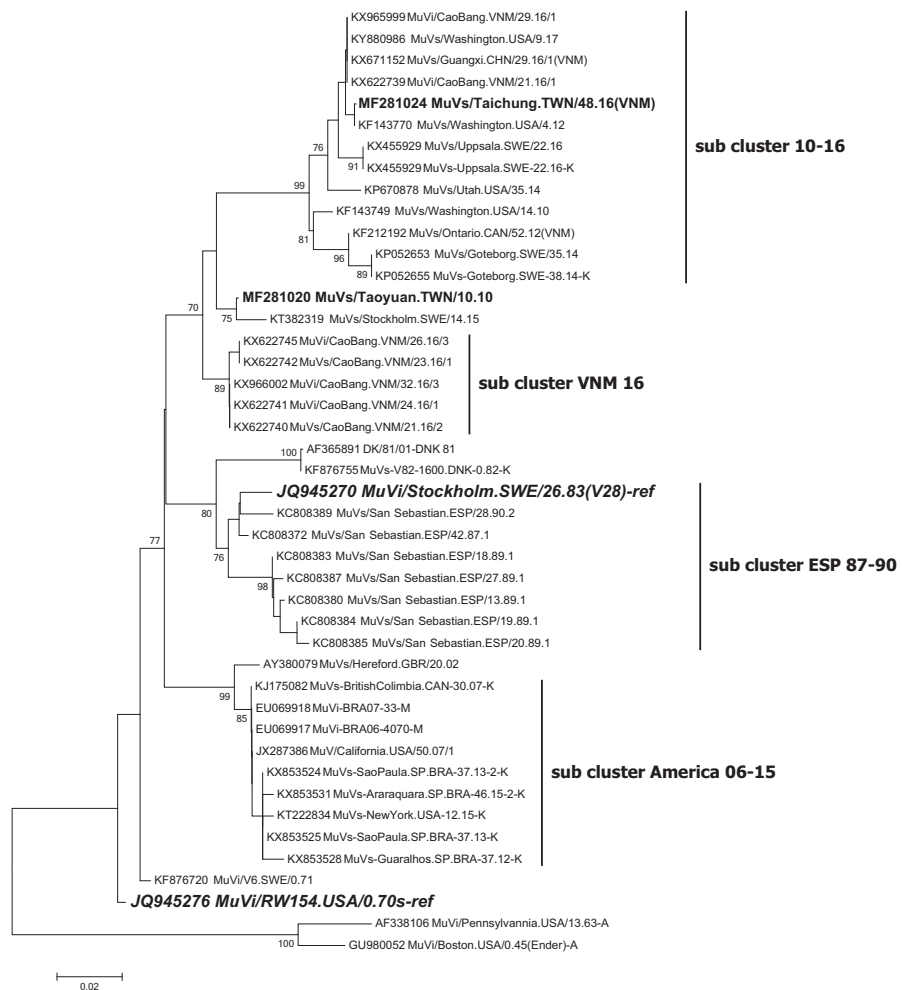


Fig. 6. Phylogenetic analysis of mumps viruses based on SH gene of Taiwan's isolates and strains isolated in other countries for genotype K, strains for reference genotype A were included as outlier of each genotype. Strain names from reference genotype are shown as bold, italic, and Taiwan's isolates are shown as bold. Bootstrap values higher than 70 in the branches are shown. The accession number and reference journal for each sequence that used to picture the sub-tree for genotype K was listed in Supplementary Table 2, the letters in parenthesis after some strain name indicated export country and shown as country abbreviation according to ISO3 code.

2010, as the incubation time averages for mumps disease was 16–18 days, whether he got mumps infection in Thailand is questionable? Furthermore, a close related strain was identified later in Sweden in 2015 (MuVs/Stockholm.SWE/14.15, KT382319), these two MuVs formed a discrete branch on phylogenetic sub tree of genotype K (Fig. 6). Sub cluster 10–16 composed of MuVs of genotype K from many countries, MuVs/Taichung.TWN/48.16 was from a Vietnamese with travel history to Vietnam, two MuVs (MuVs/Ontario.CAN/52.12, KF212192; MuVs/Guangxi.CHN/29.16/1, KX671152) identified in Canada and China individually were also imported from Vietnam, together with the other MuVs identified in Vietnam in 2016, implied genotype K MuVs circulated in Vietnam since 2012 and further spread to Sweden and the USA.

3.3. Variations in the surface protein HN

The aa constitution of surface protein of HN was translated from the complete coding sequences of HN gene (Supplementary Table 2). The N-linked glycosylation has the capacity to influence either the protein folding process or the stability of the native glycoprotein conjugate (Imperiali and O'Connor, 1999). Nine potential N-linked glycosylation site (N-X-T or N-X-S) of HN protein at aa 12–14, 127–129, 284–286, 329–331, 400–402, 448–450, 464–466, 507–509, and 514–516 were checked among 16 Taiwan's isolates. All MuVs from Taiwan contained seven potential glycosylation sites of HN protein, two genotype J strains (MuVs/Taichung.TWN/27.06[J], MuVs/Taichung.TWN/39.07[J]) and the majority of genotype G strains except MuVs/Taipei.TWN/40.15[G], lack the cytosolic glycosylation site at residues 12–14, and two genotype K strains (MuVs/Taoyuan.TWN/10.10[K], MuVs/Taichung.TWN/48.16[K]) possessed a T as the variable residue at aa 12–14 not a consensus A; two genotype J strains also lack the glycosylation site at residues 400–402, that seems to be the common characteristics of genotype J virus as genotype D and some genotype H strains. One genotype K strain (MuVs/Taichung.TWN/48.16[K]) possessed an H as the variable residue at aa 400–402 not a consensus Q.

The hemagglutinin-neuraminidase protein (HN) is the major target for the humoral immune response upon mumps virus infection (Wolinsky et al., 1985). The aa 265–288, aa 329–340 and aa 352–360 of HN protein have been known to be antigenic (Cusi et al., 2001; Kovamees et al., 1990; Orvell et al., 1997) and mutations at these location may reduce neutralizing activity induced by vaccine. Substitution at aa 265 (T265I) was found in strain MuVs/Kaohsiung.TWN/11.15 [G] imported from Japan which was similar to 3 strains isolated in Yokohama, Japan in 2010 (AB699708-AB699710) (Momoki, 2013), T275I occurred at two genotype K strain of MuVs/Taoyuan.TWN/10.10[K] and MuVs/Taichung.TWN/48.16[K] as most outbreak strains detected in Vietnam in 2016 (KX966004-KX966016), which deviated from the strains isolated in the pre-vaccine era at

1970s-1980s(JQ946040,JQ946045), or one strain reported in 2007(MF287386). T288A presented in strain of MuVs/Taipei.TWN/19.09[G] from Thailand was unique and without found in the other strains. Substitution at aa 334(V334I) in strain MuVs/Taoyuan.TWN/10.10 [K] was found at another genotype H strain MuVi/Novosibirsk.RUS/18.03[H](AY681495) and some genotype K strains related to Vietnam's outbreak in 2016 (KX966007,KX966009,KX966014 and KX966016). Substitution at aa 360(R360L) was found at one imported strain (MuVs/Taipei.TWN/46.07[G]) from Myanmar, except one genotype B strain MuVi/Himeji.JPN/24.00[B](JQ946041) presented substituted S, nearly all strain translated R at aa360.

The aa 113–130, aa 375–403 and aa 440–443 of HN protein had been identified as potentially regions for escape from neutralization by challenging wild type MuV with sera from guinea pig immunized by vaccine strain MuV (Santak et al., 2012), after inspecting sequences from Taiwan's isolates found two genotype K strains MuVs/Taoyuan.TWN/10.10[K], MuVs/Taichung.TWN/48.16[K] had substitution at aa 114(T114A) and aa 441(N441S), and one more substitution was found at aa 401(Q401H) in strain MuVs/Taichung.TWN/48.16[K]. Some genotype specific aa residues are presented in these potential antigenic epitopes, for example, aa114 (T114A) in MuVs of genotype G, the implication of the aa change requires further study.

3.4. Variations in the surface protein F

The aa constitution of surface protein of F was translated each from 14 complete coding sequences and two partial sequences of F gene (Supplementary Table 3). Five N-linked glycosylation sites of F protein at aa73-75, 182–184, 427–429, 433–435, and 457–459 were checked among 16 Taiwan's isolates. Glycosylation sites of F protein were conserved in 14 fully sequenced strains.

In a previous report it was shown that nucleotide position 271 in the F gene plays a significant role in virus pathogenesis, with G- variant cDNA clone more fusogenic in vitro but less neurovirulent in vivo than A-variant clone (Malik et al., 2007). And the S195F substitution was ever reported to be associated with a change in neurovirulence and fusion activity (Rafiefard et al., 2005). And L at position 383 of the fusion protein is responsible for the fusogenicity of wild-type MuV in B95a cells except for the Hoshino vaccine (AB470486) (Yoshida and Nakayama, 2010). All Taiwan's isolates in 5 genotypes (F, G, H, J, and K) showed concordance results of nucleotide G at position 271 and S at aa 195 of F gene, while L at aa 383 was presented in 15 isolates except for strain of MuVs/Taipei.TWN/51.06[G], which was restricted by partial F gene sequence.

4. Discussion

The global geographical distribution of mumps genotype could be pictured from 44 countries according to the genetic data collected till 2013 (Jin et al., 2015). In this study, two additional countries, including Indonesia and Myanmar had MuV genotype been reported for the first time through epidemic link to genotype J and G MuVs exportation in 2007 individually. Genotype G is the most prevalent genotype, as indicated by widely distributed in countries from six WHO regions (Jin et al., 2015). Several outbreaks were attributed to genotype G MuV including in the United Kingdom (Cui et al., 2009), the Netherlands (Gouma et al., 2016), Japan (Momoki, 2013), and in mumps resurgence in USA and Canada (Rota et al., 2009; Watson-Creed et al., 2006). Genotype F MuVs are geographic restricted to China and endemic circulation since 1995 (Cui et al., 2014; Cui et al., 2017), most of the sporadic genotype F MuVs from the other countries had epidemic link to export from China. Genotype H MuVs distributed in five of six WHO regions except the countries from the Southeast Region (SEAR), a correlation between geographic location and phylogenetic sub cluster was obvious (Fig. 4). The distribution of genotype J MuVs is relative restricted compared to genotype G, H and K, and the circulation of MuVs in cluster 2 of genotype J seem to ceased as no new sequences were found after 2002. Genotype K MuVs had never been reported from countries in WHO region of Africa or Eastern Mediterranean, the circulation of MuVs in sub cluster ESP 87–90 seemed to cease without other strains been found after 2000, the circulation of MuVs in sub cluster America 06–15 lasted, and the recent circulated MuVs were from sub cluster VNM 16 and sub cluster 10–16 including three countries, the USA, Sweden and Vietnam.

Basically, the two MuVs designated as reference in SH gene represented two branches of viruses on phylogenetic tree. Reviewed the sub tree of genotype G, H, J and K in this study, except for genotype H, the two MuVs designated as references in genotype G, J, and K were well distributed in the dichotomous branching pattern of each phylogenetic sub tree (Figs. , , and). Among them, the circulation of one reference MuV (MuVi/RW154.USA/0.70s-JQ945276) in genotype K seemed to be interrupted, and the majority of genotype K MuVs were distributed in branch contained the other reference MuV (MuVi/Stockholm.SWE/26.83-JQ945270) and formed multiple sub-clusters in relation to geographical locations. (Fig. 6). While in genotype G (Fig. 3), the MuVs of cluster 1 continued circulating, and the latest MuV in cluster 2 was found in 2012 (JQ809710) exported from Sri Lanka, the circulation of MuVs in cluster 2 needs enhance surveillance from countries in South East Asia Region. The distribution of genotype J MuVs (Fig. 5) was limited according to the sequence data available at present, and the latest sequence of genotype J MuV (KF143768) was from the USA and was distributed at sub cluster THA 06–08 in cluster 1 of genotype J, and no other MuVs in cluster 2 of genotype J were recorded since 2002, however, only limited

molecular sequences were available in countries from South East Asia Region, it's hard to tell if genotype J continuing circulated. As for MuVs in genotype H, two designated reference MuVs were distributed in cluster 1 and cluster 2 in one branch of a dichotomy branching pattern in genotype H (Fig. 4), this might result from the limited sequences available from countries of the Eastern Mediterranean and African Region when setting up standard for molecular epidemiology, once excluding sequences of the MuV in sub cluster America 12–15 that included the earliest sequence MuVs/BritishColumbia.CAN/01.12(JQ783116) imported from South Africa, and the other MuV exported from Sudan to Canada in 2007 (MuVs/Calgary.CAN/30.07-JN687468), the two reference MuVs could well distributed each at dichotomous branch pattern of genotype H (data not shown), whatever, according to the sub tree of genotype H (Fig. 4) in this study, a candidate reference MuV might be considered if the MuVs in sub cluster America 12–15 continued circulating.

Expanding molecular surveillance windows to surface protein of HN and F gene would provide additional information to discriminate different source in outbreak (Gouma et al., 2016) or tracking the aa change in potential epitopes (Cusi et al., 2001; Kovamees et al., 1990; Orvell et al., 1997) or involved in pathogenesis (Rafiefard et al., 2005; Yoshida and Nakayama, 2010), however, the structure and antigenicity of F protein seems well conversed when compared to HN protein, the deviated aa at these informative sites of HN protein were majority at strains from genotype G and genotype K. Would these changes in MuVs provide advantages for escaped from the neutralization activity in vaccinees to help it become the dominant genotype in co-circulated geographical area? What the key point in sequence change guide the direction of evolution? More intensive molecular surveillance of MuVs possible offered the solution.

This is the first report of mumps virus molecular surveillance in Taiwan, the multiple genotypes in combined with the epidemic data help to understand the chronologic and geographic distribution of mumps virus in Asia countries. Along with the advance technology in sequencing, longer sequence data other than SH gene 316 nucleotides would help to explore why outbreak occurred in highly immunized community and its relation in the process of virus evolution.

Declarations

Author contribution statement

Wen-Yueh Cheng: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Ming-Tsan Liu: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

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