Genetic Diversity of Mumps Virus in Oral Fluid Specimens: Application to Mumps Epidemiological Study

Li Jin, David W. G. Brown, Pam A. Litton, and Joanne M. White

Three hundred nine mumps virus (MuV) strains detected in the United Kingdom during 1995–2002 were characterized by partial sequencing of the small hydrophobic gene and were shown to belong to at least 6 different genotypes. A strain representing a new genotype was isolated from a seminal fluid specimen. Identical strains belonging to genotypes G and C were found to circulate for up to 3 years. One genotype H strain reappeared after an absence of 4 years. Distinct lineages (G1–G7) within genotype G were recognizable in the present study, and this level of characterization proved to be very useful for tracking MuV importations and subsequent transmission. We propose, here, a preliminary standardization of international nomenclature for genetic characterization of MuV strains, to facilitate future molecular epidemiological studies of mumps. Oral fluid (OF) specimens (n = 1441) were used to detect both anti-MuV IgM and MuV genome, and the results indicate that OF specimens can be used successfully for diagnosis and have the potential to play a valuable role in diagnosis and surveillance of mumps.

Mumps virus (MuV) is a single-strand negative-sense RNA virus of family Paramyxoviridae. Mumps is generally a childhood illness characterized by parotid gland swelling. The disease is usually mild, and approximately one-third of MuV infections are asymptomatic. However, up to 10% of patients with mumps may develop aseptic meningitis and other less frequent, but more serious, complications, such as encephalitis, deafness, orchitis, and pancreatitis, which can result in permanent disability [1].

Before the introduction of immunization, epidemics of mumps occurred in the United Kingdom at 3-year intervals [2]. Vaccination programs using measles-mumps-rubella (MMR) vaccine have greatly decreased the incidence of mumps in countries that have achieved high coverage and provide the possibility to eventually eradicate measles, mumps, and rubella worldwide. MMR vaccine was introduced in the United Kingdom in October 1988, but, in many developing countries, measles vaccination coverage is low, and mumps vaccine has not been routinely used. In countries with good coverage, imported measles and MuV infections have been recognized [3, 4]. In the United Kingdom, sporadic cases and outbreaks of mumps still occur in communities with low vaccine coverage, as well as among age groups not previously vaccinated [5].

Mumps became a notifiable disease in 1988, when MMR vaccine was introduced. Practitioners who diagnose mumps are required by statute to report all cases to the proper officer of the local government authority (usually the Consultant in Communicable Disease Control [CCDC]). There are no formal case definitions, and notifications are made on suspicion or diagnosis of clinical disease. After a school-based measles-rubella (MR) vaccination campaign in November 1994 in the United Kingdom, surveillance of measles, mumps, and rubella was enhanced and now includes IgM antibody testing of oral fluid (OF) specimens from individuals...
for whom cases of infection were reported [6]. In the United Kingdom, the percentage of reported mumps cases confirmed by testing OF for IgM antibody increased from <5% in 1995 to 38% in 2000. Individuals who are too old to have received 2 doses of MMR vaccine in the routine schedule (i.e., born between 1983 and 1992) but who are young enough to have grown up during a period of low incidence and, therefore, escaped catching mumps as a child are at particularly high risk of contracting mumps. The majority of confirmed cases occur in individuals 13–18 years old and are linked to secondary-school outbreaks, as reported elsewhere [5, 7].

By identifying the transmission pathways of the viruses, genetic characterization of wild-type (wt) viruses has become invaluable for measles control [3, 8, 9]. Similar to measles virus, MuV is serologically monotypic. However, distinct lineages of wt viruses have been described and reported to be cocirculating globally. For MuV, genotypes A–J have been reported so far. The strain diversity was identified on the basis of the nucleotide sequence of the most variable gene, the small hydrophobic (SH) gene [4, 10–14]. However, 2 groups, 1 in Japan [13] and 1 in Sweden [14], proposed 2 different branches of MuV strains as genotype J, at the same time. In our laboratory, we have previously established a genotyping scheme for MuV [4]. In this article, we (1) report the scheme’s application to the epidemiological investigation of mumps, (2) identify genetic diversity of MuV and its transmission in the United Kingdom, by use of OF specimens, and (3) provide a data set for proposing a preliminary standardization of the nomenclature for genetic characterization of wt mumps strains.

MATERIALS AND METHODS

Clinical specimens. An OF collection kit was sent by the CCDC to the notifying doctor, with a request for an OF specimen. OF specimens obtained by use of Oracol devices (Malvern Medical Developments) were mailed to our laboratory, for detection of IgM, as part of the salivary surveillance scheme in the United Kingdom [2]. In total, 1441 OF specimens obtained from individuals suspected of having mumps since 1995 were tested for mumps IgM and subsequently were tested for MuV genome. This included specimens obtained from individuals whose infections were part of a small outbreak or were sporadic cases. The majority of the patients were between 5 and 18 years old. Other specimens—including urine, semen, and blood specimens—were also tested for mumps genotypes, and serum specimens were tested for mumps IgM. In total, specimens from 1530 individuals suspected of having mumps were investigated in the present study.

Detection of mumps IgM. OF specimens were processed with 1 mL of transport medium immediately after arriving at the laboratory and then were stored at −20°C until testing. Mumps IgM was detected by use of the M antibody capture radioimmunoassay previously established in our laboratory [15].

Detection of mumps RNA. One thousand seventy-seven of the IgM-positive OF specimens and 364 of the IgM-negative OF specimens, as well as 89 specimens of other types, were then tested for mumps RNA by use of the nested reverse-transcriptase polymerase chain reaction (RT-PCR). RNA was extracted from 100 μL of the processed OF specimen by use of automatic MagnaPure extractor (Roche). A 598-nt region including the entire SH gene was amplified by RT-PCR, which was performed as described in our previous report [4]. The RT-PCR was performed in 4 geographically separate laboratories, in accordance with our standard operation procedures, to avoid the possibility of contamination.

Mumps genotyping. Sequence data were directly generated from the PCR products by use of the DyeDeoxy terminator sequencing kit (Applied Biosystems) in an automatic sequencer, as described elsewhere [4]. The nucleotide sequences were analyzed, and a phylogenetic tree was drawn by use of CLUSTAL V MegAlign software (version 5; DNASTAR), and bootstrap analysis (1000 times) was performed by use of the Neighbor-Joining method of PAUP* software (version 4.0; Sinauer Associates). The above procedures were performed essentially as has been described for measles virus [16].

RESULTS AND DISCUSSION

Correlation between time of specimen collection and the RT-PCR result. In total, 1441 OF specimens, obtained between 1995 and 2002 in the United Kingdom, were investigated in the present study. Since the OF specimens were obtained primarily for diagnostic confirmation by detection of IgM, the means of storage and transportation were not ideal for preventing degradation of viral RNA [17].

Correlation between time of specimen collection and the RT-PCR result, for IgM-positive individuals, are shown in table 1. Of 215 specimens, 133 (61.9%) were positive by RT-PCR when obtained ≤7 days after onset of disease; 22 (6.1%) of 361 specimens were positive when obtained >14 days after onset of disease. Similarly, of 364 IgM-negative specimens, 64 (31.8%)
mumps cases or that the specimens were not correctly obtained and stored [17].

**Correlation between vaccination histories of patients and results of testing for mumps IgM.** Vaccination histories were known for 1183 of the patients investigated. Table 3 shows that 474 (41.2%) of the 1150 IgM-positive individuals were vaccinated (i.e., had confirmed primary vaccination), which suggests that, after having received 1 dose of MMR vaccine, these patients experienced vaccine failure or that, in the 1994 school vaccination campaign, these patients were misidentified as having received MMR vaccine rather than MR vaccine. The efficacy of the mumps component of MMR vaccine is thought to be ~85%; therefore, these results justify the strategy, implemented in 1996, of offering a second dose of MMR vaccine to children 3–5 years old. In addition, 11 of 12 patients who were recently (2–3 weeks before onset of disease) vaccinated were found to be IgM positive. Five of these were also positive by RT-PCR: 4 were found to have genotype G5 [19], and 1 was found to have genotype D3. The IgM-negative individual was found to have genotype F. None was found by sequencing to have the vaccine strain, suggesting that these patients were exposed to wt mumps around the time of vaccination. In general, of the patients for whom infection was confirmed by detection of mumps IgM between 1995 and 2002 and whose vaccination status is known, 40% had not received any MMR vaccine, 52% had received only 1 dose, and 4% had received 2 doses, emphasizing the need to include a second dose of MMR in the schedule.

**Proposals for designation of new genotypes and subgenotypes (G1–G7).** Genotyping based on the sequence variation of the SH gene has been established, and genotypes A–J have been reported so far [4, 10–14]. The genotype J strains designated by the groups in Japan [13] and in Sweden [14] were reanalyzed, and, with the permission of the authors, in the present study, we have reclassified the Denmark strains as genotype K (figure 1). MuV strains RW/US80 [20] and DK/81/01 [14] were most closely related, and both could be the reference strains of genotype K, although there are only 271 nt available for strain DMK/81/01 (figure 1). Strains MP94H/Jap94 [13] and Loug1UK97 [4] could be the reference strains

<table>
<thead>
<tr>
<th>Days after onset of disease</th>
<th>Positive</th>
<th>1–7</th>
<th>8–14</th>
<th>&gt;14</th>
<th>Not known</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>64</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>137</td>
<td>127</td>
<td>15</td>
<td>14</td>
<td>293</td>
<td></td>
</tr>
<tr>
<td>Total (%) positive</td>
<td>201 (11.8)</td>
<td>130 (2.3)</td>
<td>16 (6.2)</td>
<td>17 (17.6)</td>
<td>364</td>
<td></td>
</tr>
</tbody>
</table>

**NOTE.** Data are no. of patients.

Table 2. Correlation between time of oral fluid specimen collection and reverse-transcriptase polymerase chain reaction (RT-PCR) results, for IgM-negative individuals.

<table>
<thead>
<tr>
<th>Result of testing for IgM</th>
<th>Vaccinated</th>
<th>Nonvaccinated</th>
<th>Not known</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>474</td>
<td>400</td>
<td>276</td>
<td>1150</td>
</tr>
<tr>
<td>Negative (%) positive by PCR</td>
<td>42</td>
<td>10</td>
<td>19</td>
<td>71 (4.6)</td>
</tr>
<tr>
<td>Negative (%) negative by PCR</td>
<td>159</td>
<td>98</td>
<td>82</td>
<td>309 (20.2)</td>
</tr>
<tr>
<td>Total</td>
<td>675</td>
<td>508</td>
<td>347</td>
<td>1530</td>
</tr>
</tbody>
</table>

**NOTE.** Data are no. of patients. PCR, polymerase chain reaction.
of genotype J (figure 1). The results of the present study, together with those from our previous report [4], have provided further evidence for the requirement to set up an international standardization of the nomenclature for genetic characterization of wt mumps strains.

Seven genotypes (C, D, F, G, H, J, and K) were identified from 309 specimens with positive PCR results that were obtained during 1995–2002, and 226 of these belonged to genotype G. On the basis of previous reports [10, 11, 21], a preliminary nomenclature was proposed, in which sequence diversity of >8% in the SH gene was used to designate new genotypes. However, this might need modification, since more MuVs have been characterized. Seven distinct lineages of genotype G were found to be circulating in the United Kingdom (table 4) and were designated as G1–G7 in our laboratory (figure 1 and figure 2). The divergences between these subgenotypes were 1.1%–4.3%, on the basis of the 598-nt sequence generated in the present study (table 4), and 0.6%–3.2%, compared with those between genotypes (>5%), on the basis of the 318-nt sequence of the SH gene [4]. These differences were reliably detected and useful for studying transmission patterns.

The mumps strain detected from a seminal fluid specimen from a patient with orchitis [22] revealed divergences of 7.7%–19.3%, on the basis of the 318-nt sequence of the SH gene (figure 1), and 3.6%–37.7%, on the basis of 57 aa of the SH gene. We propose that this strain represents a new genotype when a similar strain is discovered. Representative strains of all genotypes and subgenotypes were included in the phylogenetic tree (figure 1).

**Chronological distribution of mumps genotypes.** The majority of mumps cases were linked to secondary-school outbreaks, affecting individuals 13–19 years old. Three hundred nine PCR products, including those obtained from a range of specimens (urine, blood, and semen specimens), were selected from representative cases and subsequently were sequenced for genotyping. Specimens obtained from siblings of patients during outbreaks or from geographically and temporally related cases, which were suspected of being similar genotypes, were screened by use of amplification refractory mutation system–EIA, a nonsequencing genotyping method [23]. The chronological distribution of mumps genotypes, on the basis of these 309 cases, is summarized in table 4. There were only 11 mumps strains identified before 1988, when the vaccination program was initiated in the United Kingdom, and all of them belonged
to genotype C [10], indicating that genotype C might have been predominant in the prevaccination era in the United Kingdom.

Eight strains identified during 1989 and 1991, in a previous study [10], were reanalyzed on the basis of the current, updated genotyping scheme [4]: 2 were found to be genotype B, 4 were found to be genotype C, 1 was found to be genotype G (G1), and 1 was found to be genotype H. Multiple genotypes were identified during 1995–2002, including genotypes C, D, F, G (G1–G7), H, J, and K (table 4). The results revealed a diversity of strains consistent with cocirculation of multiple genotypes of mumps, which has also been reported in other studies [13, 14]. None of these strains is related to the current vaccine strain used in the United Kingdom, JL/US63, which belongs to genotype A. This raises the question of whether the current vaccine would protect populations from infection by any other mumps genotypes. wt strains of genotype A have not been detected in the United Kingdom before or after the introduction of mumps vaccination (table 4). The effectiveness of the current vaccine strain has suggested that vaccine-induced immunity protects against other genotypes. However, 1 genotype A strain (SBL-1), which was originally isolated in Sweden, was found to be endemic in Sweden from 1971 to 1999 and did not show high neutralization titers against the antibody induced by vaccine strain JL in MMR vaccines [24]. Ongoing surveillance is necessary.

Transmission of MuV strains and links to importations. In addition to the 4 outbreaks reported elsewhere [4], another 6 outbreaks or geographically linked transmissions were investigated in the present study. One genotype G (G2) strain circulated in Northern Ireland between December 1999 and October 2001, and an identical strain was found in 23 cases. A different G2 strain was endemic in the northwest region of England, starting in June 2000 and ending in July 2002, and 84 identical strains were identified during this period. On the basis of the 598-nt, rather than the 318-nt, sequence, the divergence between those 2 G2 strains was 1.2%, compared with the divergences between subgenotype G strains (1.1%–4.3%). Identical G4, G5, and G6 strains were detected in the northwest during January–May 2000, in the West Midlands during March–July 2000, and in the Manchester and Liverpool areas during February–August 2001 (figure 2).

Genotype C strains reappeared in the United Kingdom from 1998 to 2000, after a gap of at least 3 years (no data available for 1992–1994), and were identified from the outbreak in London [5]. In 2000, small divergences were found in the genotype C strains detected in specimens from individuals whose infections were sporadic cases.

Divergent genotype H strains have been found to be widely distributed throughout the United Kingdom in most years since 1995. However, 1 genotype H strain was detected in 1998 and again 4 years later, in 2002, without differences. One school-related outbreak in the northwest in 1999 was due to a genotype F strain (table 4), and an identical strain was detected in specimens from 13 of 14 individuals. The index case and the source of this outbreak remain unknown. Genotype F strains had previously been found only in China [21]. Interestingly, this genotype F strain has not been found elsewhere in the United Kingdom since then, suggesting its possible link with importation, although there was no clear contact history with the index case. The independent circulation of these characterized strains suggests that subgenotyping is valuable to assist epidemiological investigations of virus transmission. The present study has shown that multiple genotypes or strains are repeatedly introduced into the United Kingdom, as reported elsewhere [4].

In the present study, several cases of mumps infection were clearly due to importation. A genotype D strain was detected

Table 4. Chronological distribution of mumps genotypes in the United Kingdom.

<table>
<thead>
<tr>
<th>Yeara</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>F</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>G4</th>
<th>G5</th>
<th>G6</th>
<th>G7</th>
<th>New</th>
<th>H</th>
<th>J</th>
<th>K</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1975–1988</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11b</td>
</tr>
<tr>
<td>1989–1991</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8b</td>
</tr>
<tr>
<td>1995</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>1996</td>
<td>1</td>
<td>1</td>
<td></td>
<td>2</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>1997</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7</td>
</tr>
<tr>
<td>1998</td>
<td>16</td>
<td>1</td>
<td></td>
<td>4</td>
<td>1</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>1999</td>
<td>22</td>
<td>2</td>
<td>14</td>
<td>7</td>
<td>7</td>
<td>5</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>58</td>
</tr>
<tr>
<td>2000</td>
<td>5</td>
<td></td>
<td>24</td>
<td>14</td>
<td>11</td>
<td>15</td>
<td>2</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td>74</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2001</td>
<td>1</td>
<td>1</td>
<td>59</td>
<td>1</td>
<td>24</td>
<td>5</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>95</td>
</tr>
<tr>
<td>2002</td>
<td>1</td>
<td>37</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>44</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>0</td>
<td>43</td>
<td>9</td>
<td>14</td>
<td>2</td>
<td>128</td>
<td>7</td>
<td>15</td>
<td>20</td>
<td>47</td>
<td>7</td>
<td>14</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>309</td>
</tr>
</tbody>
</table>

a Data are not available for 1992–1994.

b Data from previous published report [10].
Figure 2. Phylogenetic tree presenting the mumps genotypes detected in the United Kingdom during 1998 and October 2002. Genotypes are indicated on the branches, and identical strains are boxed as groups. The tree was drawn on the basis of a 598-nt sequence of the entire polymerase chain reaction–generated “fusion–small hydrophobic–hemagglutinin-neuraminidase” region by use of the CLUSTAL V MegAlign program (DNASTAR).

from a patient who returned from Italy in 2001, and a G5 strain was detected from a patient who had contact with visitors from Bangladesh in 2002. There is no knowledge of endemic strains in those countries, and, therefore, the present study has indirectly provided evidence of mumps genotypes circulating in those countries. The application of genotyping directly to clinical specimens will lead to a more complete picture of MuV genotype distribution worldwide, which will contribute to the surveillance of mumps.

Mumps genotypes and associations with clinical symptoms. Most of the patients investigated in the present study showed classical symptoms of mumps infection (fever and parotitis) with no complications, such as meningitis, pancreatitis, and orchitis, and individual genotypes were not clearly linked
with particular symptoms. Three patients, 1 with meningitis and 2 with orchitis, were investigated further: meningitis was found to be due to a genotype G2 strain detected in the cerebrospinal fluid specimen in 2002, which was identical to the majority of the G2 strains detected in 2002; orchitis was found to be due to 2 different MuV genotypes. One was genotype D, detected from an OF specimen, and the other was considered to represent a new genotype strain [22], in which the MuV genome was detected from 2 seminal fluid specimens obtained 2 and 4 weeks after onset of disease. An identical sequence was found, suggesting that virus shedding via seminal fluid could be prolonged up to 4 weeks and that genetic heterogeneity, compared with other genotypes, was not due to prolonged replication. There were no other specimens and no clear record of travel history or foreign contact available for this patient.

**Mutations detected in the hemagglutinin-neuraminidase (HN) gene.** The 598-nt sequence generated for mumps strains in the present study encompasses the noncoding regions between the fusion, SH, and HN genes and the predicted 57 aa in the SH gene and 48 aa at the amino (N)-terminal of HN gene. The SH protein has an N-terminal stretch of 25 hydrophobic residues, which could act as a membrane anchor region [25]. There are 9 potential N-glycosylation sites on the HN, which are important antigen recognition sites [26], and 1 is included in the 598-nt sequence. In the present study, this N-glycosylation site (Asn-Ala-Thr), located at aa 12–14 of the HN, was detected in MuV strains of genotypes D, F, and H and in 1 strain of genotype G. However, because of an A→G substitution at the second of the triplet AAT (Asn)–AGT (Ser) (in 224 cases) and 2 A→G changes AAT-GGT (Gly) (in 1 case), this site did not exist in most of the genotype G strains, and, because of an A→G mutation AAT-GAT (Asp), it did not exist in all of the genotype C strains. Similar substitutions occur in most of the genotype G strains and also was found in the sequence of the genotype G. However, because of an A→G substitution at the second of the triplet AAT (Asn)–AGT (Ser) (in 224 cases) and 2 A→G changes AAT-GGT (Gly) (in 1 case), this site did not exist in most of the genotype G strains, and, because of an A→G mutation AAT-GAT (Asp), it did not exist in all of the genotype C strains. Similar substitutions occur in most of the genotype G strains and also was found in the sequence of the new genotype strain detected in the seminal fluid specimen (GenBank accession no. AY380077). Biological significances of the coding changes in the SH and the HN genes require further investigation.

In conclusion, this study has shown that OF specimens are very reliable for detection of mumps IgM antibody and viral RNA by nested RT-PCR and, in the future, have the potential to replace blood specimens for diagnosis. The increasing diversity of viruses detected in a relatively small number of cases of mumps and the evidence of imported cases into the United Kingdom are similar to those for measles [9]. Monitoring geographical and temporal distribution of mumps genotypes in the United Kingdom, against a background of high herd immunity, is essential for surveillance of mumps and contributes to programs for the control and elimination of mumps disease. Analysis at the level of subgenotypes (G1–G7) has proven to be very useful in monitoring evolution and tracking transmission of MuV strains. GenBank accession numbers for sequence data generated in the present study are AY380062–AY380079.

**Acknowledgments**

We thank Stuart Beard, Richard Harrison, and Rashpal Hunjan, for their technical support; and C. Örvell for granting permission to rename the genotype J strain that his group appointed, to genotype K, to avoid confusion caused by its duplication.

**References**

17. Jin L, Yves AJ, Brown DWG. The detection of measles, mumps and


