Mumps clinical diagnostic uncertainty

Fabio Magurano 1, Melissa Baggieri 1, Antonella Marchi 1, Paola Bucci 1, Giovanni Rezza 2, Loredana Nicoletti 1

1 National Reference Laboratory for Measles and Rubella, Department of Infectious Diseases, National Institute of Health, Rome, Italy
2 Department of Infectious Diseases, National Institute of Health, Rome, Italy

Correspondence: Fabio Magurano, Department of Infectious Diseases, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy. Tel./fax: +39 06 49902448, e-mail: fabio.magurano@iss.it

Background: During recent years, various mumps outbreaks have occurred among populations vaccinated for mumps worldwide. In Italy, improving routine coverage with two doses of measles, mumps and rubella (MMR) vaccine is one of the key strategies to eliminate measles and rubella. To monitor the effect of the vaccination programme on the population, the surveillance of these vaccine-preventable diseases has been implemented. This provided the opportunity to evaluate the accuracy of the clinical diagnosis of those diseases, including mumps. In fact, vaccinated children may develop a variety of diseases caused by a series of different viruses [Epstein-Barr virus (EBV), parainfluenza virus types 1–3, adenoviruses, herpes virus and parvovirus B19] whose symptoms (i.e. swelling of parotid glands) may mimic mumps. For this reason, laboratory diagnosis is essential to confirm clinical suspicion.

Methods: The accuracy of clinical diagnosis of mumps was evaluated by differential diagnosis on EBV in Italy, a country at low incidence of mumps. This retrospective study investigated whether the etiology of 131 suspected mumps cases with a negative molecular/serological result for mumps virus, obtained from 2007 to 2016, were due to EBV, in order to establish a diagnosis. Results: Differential diagnosis revealed a EBV positivity rate of 19.8% and all cases were caused by EBV type 1. Conclusions: This study confirms the importance of a lab based differential diagnosis that can discriminate between different infectious diseases presenting with symptoms suggestive of mumps and, in particular, emphasize the importance to discriminate between mumps and EBV-related mononucleosis.
Clinical criteria
Fever and at least two of the following: sudden onset of unilateral or bilateral tender swelling of the parotid or other salivary glands without other apparent cause or orchitis or meningitis.

Laboratory criteria
At least two of the following (i) isolation of mumps virus from a clinical specimen; (ii) detection of mumps virus nucleic acid; (iii) MuV specific antibody response characteristic for acute infection in serum or saliva.

Epidemiological criteria
An epidemiological link by human-to-human transmission.
Case classification includes ‘possible case’ (any person meeting the clinical criteria), ‘probable case’ (any person meeting the clinical criteria and with an epidemiological link), ‘confirmed case’ (any person not recently vaccinated and meeting the clinical and the laboratory criteria).

Study population and clinical samples
During the period between June 2007 and 2016, oral and/or blood samples of 193 suspected cases of mumps were sent to the NRL from various Italian regions for laboratory confirmation of the clinical diagnosis. All these were clinically compatible and sporadic cases, not linked to outbreak settings. The vaccination status was known for 190/193 patients: 42 had never received a vaccine against mumps (41.6%), 79 were vaccinated with 1 dose (36.3%), 69 with 2 doses (22.1%).
Mumps virus genome can be detected from oral fluid within the first week after symptoms onset. Oral fluid from suspected mumps cases were tested for mumps by RT Real-Time PCR, while blood samples were tested for specific IgM anti-mumps detection by Elisa. Oral fluid samples still available of those negative patients were further tested for EBV by PCR.

Serological diagnosis for mumps
The detection of anti-mumps IgM was performed with the Enzygnost Anti-Parotitis Virus/IgM kit (Dade/Behring, Siemens) on blood samples collected and treated as previously described in. 17

Molecular detection of mumps and EBV
RNA and DNA were extracted from oral fluid specimens using QIAamp Viral RNA Kit and QIAamp DNA Mini Kit (Qiagen), respectively, according to the manufacturer’s instructions.
A 7 μl aliquot of RNA was used for a reverse transcription PCR Real-time with the RealTime Ready RNA Virus Master kit (Roche) according to CDC’s indications. 18
A portion of 169 bp of the BXLF1 gene of EBV was amplified by PCR. The reaction was performed with PCR Supermix (Invitrogen), 10 pmol of each forward (EBV1 5' - GGGCGCAATCTGGTTTAG-3', position 143 411) and reverse primers (EBV2 5' - CCGGGGACCACCATAGT-3', position 143 379), and 3 μl of extracted DNA. 19
The cycling conditions consisted of an initial denaturation of 10 s at 95°C, followed by 45 cycles of 40 s at 95°C, 1 min at 58°C and 40 s at 72°C and a final extension of 5 min at 72°C.

Genetic analysis
Samples positive for MuV were further amplified for genotyping by PCR followed by a Nested PCR on the MuV SH gene, using the SuperScript One-Step RT-PCR with PlatinumR Taq System and PCR Supermix kits (Invitrogen), respectively. Before sequencing, PCR products were purified with the QIAquick PCR Purification Kit (QIAGEN) and sequencing reactions performed by Macrogen Inc. (Seoul, South Korea). Nucleotide sequences were aligned with sequences of the reference strains and with those that showed a high percentage of identity after Blast analysis, using CLUSTAL W (BioEdit) software. 20 The Bayesian Information Criterion was used to determine the model of nucleotide substitution that best fit the data using the selection tool available in MEGAl. 21 Evolutionary analyses were conducted using the maximum likelihood method based on the Tamura 3-parameter (T92) model and evolutionary rates among sites were modelled by a discrete Gamma distribution (+G).
Samples positive for EBV were tested by PCR to amplify a portion of the gene EBNA3C in order to discriminate between EBV genotype type 1 or type 2. 22 PCR was performed with PCR Supermix (Invitrogen) with 5 min at 95°C, followed by 35 cycles of 45 s at 95°C, 45 s at 56°C and 1 min at 72°C and 10 min at 72°C. Amplicons were analysed by electrophoresis on 1.5% agarose gel and gel-red staining.

Results
From June 2007 to 2016, 148 oral fluid and 169 blood samples from a total of 193 patients with suspect mumps were collected and tested at the NRL. As reported in table 1, 11/193 (5.7%) patients were found positive for MuV infection either by serological or molecular assay, and 182 were negative. Three cases were positive by IgM serology but negative by PCR probably due a bad sampling. Detailed results and vaccination status for each positive patient are reported in table 2. Vaccination status was available for 9 out of 11 positive cases: 5 mumps infected patients had received one dose of MMR vaccine, 2 patients had received two doses and 2 were not vaccinated. For five patients, it was possible to calculate the time elapsed after vaccination (ranging from 2.5 to 13 years). For those negative cases, 66.5% (121/182) had received at least one dose of vaccine against mumps while 20.9% (38/182) were not vaccinated.
Phylogenetic analysis was performed on 5 MuV sequences obtained from samples positive in PCR. As shown in figure 1, four strains belonged to genotype G (MuVs/Salerno.ITA/4.14/, MuVs/Bolzano.ITA/18.11/, MuVs/Livorno.ITA/24.14/) and one to genotype H (MuVs/Livorno.ITA/41.07/). Sequences were deposited in GenBank database under accession numbers KX518652, KX518653, KX518654, KX518655, KX518656.
WTH data show that genotype G has been reported in Europe, North America, South-East Asia, while genotype H has been reported also from South America and Africa. BLAST analysis showed that the same strain identified in Salerno in 2014 circulated in Europe in 2012 and 2013 and in USA in 2016. The strain that circulated in Bolzano in 2011 was also identified in Germany in the same year. No strains identical to those identified in Livorno in 2014 and 2016 have been ever reported. In addition, the unique strain belonging to the genotype H, identified in Livorno in 2007, did not show identity with any other strains after BLAST analysis.
Oral fluid samples available for 131 out of 182 mumps negative cases were further tested for EBV by PCR; of them, 26 were found positive (positivity rate of 19.8%) for viral DNA (table 1).

Beside genetic analysis on mumps strains, EBV positive samples were tested by PCR to distinguish between genotypes type 1 or 2, and all of them belonged to genotype 1.
The incidence trend of new cases of mumps in Italy from 1996 to 2014 shows a series of oscillations, with a maximum of almost 65 000 cases reported in 1996 (figure 2). Since 1999, the incidence of mumps declined to a minimum number of 191 cases reported in 2014. This decline was probably due to MMR vaccination campaigns. Studies established that the effectiveness of any MMR vaccination in patients with a history at least one MMR vaccination adjusted for age, sex and general practice was 69% (95% CI: 41–84% 24,25 and because of the low effectiveness of the mumps campaigns.
MMR vaccine component, several outbreaks occurred in Europe.\textsuperscript{26,27}

Also, the decreased efficiency of the surveillance system, leading to a low notification rate, was likely to contribute to the low number of cases reported in Italy in the last years.

The standard clinical case definition of mumps used for surveillance activities consisted in 'acute onset of unilateral or bilateral swelling of the parotid or other salivary glands lasting two or more days without any other apparent cause'.\textsuperscript{2} However, although parotitis is indeed the hallmark of mumps, there are cases in which salivary-gland swelling is not apparent, especially in individuals with mumps meningitis, many of whom do not present detectable salivary-gland enlargement.\textsuperscript{28,29} Moreover, other infectious agents may also cause salivary-gland swelling. The effect of such alternative aetiologies greatly reduces the positive predictive value of a clinical diagnosis when the disease incidence is low.\textsuperscript{30}

This study reports results from the differential diagnosis of mumps with EBV-related mononucleosis provides information on the specificity of the clinical diagnosis of mumps, suggesting the importance of laboratory confirmation.

### Table 1

<table>
<thead>
<tr>
<th>Year</th>
<th>Mumps pos</th>
<th>Mumps neg</th>
<th>Mumps tested</th>
<th>EBV pos</th>
<th>EBV neg</th>
<th>EBV tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007</td>
<td>4</td>
<td>45</td>
<td>49</td>
<td>12</td>
<td>33</td>
<td>45</td>
</tr>
<tr>
<td>2008</td>
<td>1</td>
<td>41</td>
<td>42</td>
<td>10</td>
<td>30</td>
<td>40</td>
</tr>
<tr>
<td>2009</td>
<td>1</td>
<td>13</td>
<td>14</td>
<td>4</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>2010</td>
<td>0</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2011</td>
<td>1</td>
<td>14</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2012</td>
<td>3</td>
<td>25</td>
<td>25</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2013</td>
<td>0</td>
<td>17</td>
<td>17</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2014</td>
<td>1</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2015</td>
<td>1</td>
<td>9</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2016</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>49</td>
<td>182</td>
<td>193</td>
<td>26</td>
<td>105</td>
<td>131</td>
</tr>
</tbody>
</table>

### Table 2

<table>
<thead>
<tr>
<th>Year</th>
<th>PCR</th>
<th>IgM</th>
<th>Genotype</th>
<th>Age</th>
<th>Vaccination status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pt 229</td>
<td>2007</td>
<td>POS</td>
<td>–</td>
<td>17 months</td>
<td>1 dose</td>
</tr>
<tr>
<td>Pt 256</td>
<td>2007</td>
<td>NEG</td>
<td>POS</td>
<td>4 years</td>
<td>1 dose</td>
</tr>
<tr>
<td>Pt 419</td>
<td>2007</td>
<td>POS</td>
<td>POS</td>
<td>13 years</td>
<td>1 dose</td>
</tr>
<tr>
<td>Pt 427</td>
<td>2007</td>
<td>–</td>
<td>POS</td>
<td>13 years</td>
<td>NA</td>
</tr>
<tr>
<td>Pt 1368</td>
<td>2008</td>
<td>–</td>
<td>POS</td>
<td>46 years</td>
<td>Not vaccinated</td>
</tr>
<tr>
<td>Pt 1806</td>
<td>2009</td>
<td>NEG</td>
<td>POS</td>
<td>6 years</td>
<td>1 dose</td>
</tr>
<tr>
<td>Pt 2322</td>
<td>2011</td>
<td>POS</td>
<td>–</td>
<td>15 years</td>
<td>1 dose</td>
</tr>
<tr>
<td>Pt 3060</td>
<td>2014</td>
<td>POS</td>
<td>BL</td>
<td>18 years</td>
<td>2 doses</td>
</tr>
<tr>
<td>Pt 3143</td>
<td>2014</td>
<td>POS</td>
<td>POS</td>
<td>19 years</td>
<td>NA</td>
</tr>
<tr>
<td>Pt 3196</td>
<td>2014</td>
<td>NEG</td>
<td>POS</td>
<td>6 years</td>
<td>2 DOSES</td>
</tr>
<tr>
<td>Pt 3398</td>
<td>2016</td>
<td>POS</td>
<td>POS</td>
<td>29 years</td>
<td>Not vaccinated</td>
</tr>
</tbody>
</table>

NA, not applicable.
Our findings show that the specificity of the case-definition of mumps is low. Studies conducted in other areas of the world provided similar results. In a study conducted in Victoria, Australia, only 7 (9%) of 74 cases clinically diagnosed as mumps parotitis could be confirmed by serology; 7 (16%) of 43 laboratory-rejected cases were positive for EBV using serological testing. In a study conducted in Finland, on 601 acutely ill children presenting mumps-like symptoms but seronegative for mumps, the most commonly identified viral agents were the EBV (7%), parainfluenza virus (4%) and adenovirus (3%). These studies highlight the importance of laboratory confirmation in diagnosing mumps, especially under non-outbreak conditions.

Discussion

According to WHO, introduction of routine mumps vaccination, such as other prophylactic options, should be a high priority. Most European health systems provide mumps vaccine in combination with MMR, with a two-dose vaccination schedule, free of charge, and some 120 countries have introduced vaccination against mumps in their national immunization programmes. To date, countries such as Finland or Sweden have completely eradicated mumps from their national territory. Actions should be implemented to encourage practitioners to collect oral and blood samples from mumps suspected cases and to submit these samples to the NRL or other reference labs that performs mumps virus PCR and serology. This is of special importance when the patient is vaccinated and a primary or secondary vaccination failure is suspected, being important both for individual patients and for monitoring the outcome of vaccination programmes. About that, our study revealed that three patients positive for mumps had been vaccinated before the introduction (in 2001) of the more efficient component Urabe AM 9 in the MMR vaccine in spite of the Rubini strain, responsible for some vaccine failure.

In conclusion, the results of this study confirm the importance of a lab-based differential diagnosis that can discriminate between different infectious diseases presenting with symptoms suggestive of mumps and emphasize the importance to discriminate between mumps and EBV-related mononucleosis. Finally, the large proportion of negative results suggests that other viral infections are involved in the genesis of mumps-like syndromes.

Acknowledgements

We wish to thank Dr C. Fortuna and Mrs E. Benedetti for technical support, and the staff of Italian Regional and Local Health Authorities for providing clinical specimens.

Funding

This work was partially funded by the Italian Ministry of Health grant CCM 2015-6M21.

Conflicts of interest: None declared.

Key points

- The specificity of the case-definition of mumps is low and a large number of viral infections are involved in the genesis of mumps-like syndromes such as Epstein-Barr virus.
- A lab-based differential diagnosis is essential to discriminate between different infectious diseases, especially for the large proportion of mumps negative cases.
- Low efficiency of the surveillance system for mumps, leading to a low notification rate, contribute to the low number of cases reported in Italy in the last years.

References


