Molecular epidemiology of mumps viruses in the Netherlands, 2017-2019

Rogier Bodewes*, Linda Reijnen, Jeroen Kerkhof, Jeroen Cremer, Dennis Schmitz, Rob van Binnendijk, Irene K. Veldhuijzen

Centre for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands

* rogier.bodewes@rivm.nl

Abstract

Mumps cases continue to occur, also in countries with a relatively high vaccination rate. The last major outbreaks of mumps in the Netherlands were in 2009–2012 and thereafter, only small clusters and single cases were reported. Molecular epidemiology can provide insights in the circulation of mumps viruses. The aims of the present study were to analyze the molecular epidemiology of mumps viruses in the Netherlands in 2017–2019 and to compare the phylogenetic trees built from sequence data of near complete mumps virus genomes or from the SH gene and non-coding regions (SH+NCRs). To this end, Sanger sequence data from SH+NCRs were analyzed from 82 mumps genotype G viruses. In addition, near complete genomes were obtained from 10 mumps virus isolates using next-generation sequencing. Analysis of SH+NCRs sequences of mumps genotype G viruses revealed the presence of two major genetic lineages in the Netherlands, which was confirmed by analysis of near complete genomes. Comparison of phylogenetic trees built with SH+NCRs or near complete genomes indicated that the topology was similar, while somewhat longer branches were present in the phylogenetic tree with near complete genomes. These results confirm that analysis of SH+NCRs sequence data is a useful approach for molecular surveillance. Furthermore, data from recent mumps genotype G viruses might indicate (intermittent) circulation of mumps genotype G viruses in the Netherlands in 2017–2019.

Introduction

Mumps viruses are single stranded negative-sense RNA viruses belonging to the genus Orthorubulavirus of the family of Paramyxoviridae. Infection of humans with mumps virus results in acute illness which is usually characterized by a temporary unilateral or bilateral parotitis. Occasionally, mumps virus infections can result in serious complications [1, 2], but infections with mumps virus also often do not result in recognized clinical signs [1, 3].

In 1987, vaccination against mumps virus was implemented in the National Immunization Program in the Netherlands. Although this resulted in a rapid decline of mumps cases, in recent years multiple outbreaks occurred mainly among vaccinated young adults [4, 5]. The last major outbreaks of mumps in the Netherlands were in 2009–2012 [5]. Thereafter, only
small clusters and single cases were reported [6]. From 2013 until 2020, multiple small local outbreaks and individual mumps cases were reported in the Netherlands [6].

Molecular epidemiology can provide insights in the circulation of mumps viruses. Sequencing of the SH gene and adjacent non-coding regions provides information about the genotype and some information of circulating strains [7, 8]. Increasing the molecular resolution by obtaining additional sequence data of mumps viruses has proven useful in defining different transmission chains and local clusters [7, 9, 10]. In this context, sequencing of the hemagglutinin-neuraminidase protein gene (HN) and fusion protein gene (F) provides similar sequence resolution compared to sequencing of the sum of three non-coding regions (NCRs), present between the nucleocapsid protein and phosphoprotein (N-P), between phosphoprotein and matrix protein (P-M) and between matrix protein gene and F gene (M-F). However, sequencing of the complete genome might provide the more complete resolution that is necessary to identify exact transmission chains and it has the advantage of easy comparison with other mumps viruses on a global scale [11–15].

The aims of the present study were to analyze the molecular epidemiology of mumps viruses in the Netherlands in 2017–2019 and to compare phylogenetic trees built using near complete genomes with SH+NCRs. To this end, we investigated all typable specimens generated from isolated viruses of molecular confirmed mumps cases in the Netherlands from 2017 to 2019.

Materials and methods
Sample collection, molecular detection, Sanger sequencing and virus isolation
Clinical samples (oral fluid, throat swab and/or urine) from suspected mumps cases (acute unilateral or bilateral parotitis) were submitted by municipal health services or clinical microbiological laboratories in the Netherlands via regular mail at room temperature to the National Institute for Public Health and the Environment (RIVM) for molecular diagnostics. Upon arrival at RIVM, samples were stored at +4°C until further processing. RNA was extracted by automated extraction using the MagNA Pure 96 extraction (Roche Diagnostics) with DNA and Viral NA Small Volume kit and molecular detection of mumps virus RNA was performed by real time quantitative PCR (RT-qPCR) using primers as described previously [16]. In addition, clinical materials from RT-qPCR-confirmed mumps cases, that were notified under the Public Health Act in the Netherlands, were send to the RIVM for molecular surveillance by clinical microbiological laboratories in the Netherlands via regular mail at room temperature. Sequencing of the SH region and NCRs between the N and P, P and M and M and F genes was performed on nucleic acids extracted from clinical materials using the OneStep RT-PCR kit (Qiagen) as described previously [7, 8]. Sanger sequencing was performed at BaseClear (Leiden, the Netherlands). Molecular surveillance of mumps viruses, including this study, is part of the Public Health Act in the Netherlands.

In total 73 clinical materials (oral fluid, throat swabs and urine) were used for virus isolation. Confluent Vero cells (ECACC 84113001) in 24 wells plates (Greiner Bio-One) were inoculated with clinical material (5μl oral fluid, 10μl urine or 50μl throat swab medium) and cells were incubated for one hour at 37°C/5% CO₂. Subsequently, 1 ml of Dulbecco’s Modified Eagle Medium (DMEM, Gibco) supplemented with 100U/ml Pen-Strep (Lonza), 2 mM L-Glutamine (Lonza), 12.5 μg/ml Amphotericin B (Biowest) and 24 units/ml Nystatin suspension (Sigma) was added and plates were incubated at 37°C/5% CO₂. Wells were checked daily for the presence of cytopathic effect (CPE). If no CPE was present after five days, cells were trypsinized (0.25% Trypsin-EDTA(1X) Gibco) and subsequently about 1/3 of trypsinized cells were
transferred to another well and fresh medium was added. Again, cells were checked daily for
the presence of CPE. If no CPE was present for 12 days after the start of the first incubation,
culture was considered negative. If CPE was detected, medium was harvested and subsequently
passaged one or two times over confluent Vero cells to produce a virus stock in a T25 cm²
flask or T75 cm² flask (Corning) resulting in 8 or 25 ml cell culture supernatant medium con-
taining mumps virus.

Next-generation sequencing

Mumps virus isolates were processed for full genome sequencing using a viral metagenomics
approach. To this end, 200 µl of each cell culture supernatant was pretreated and processed for
next-generation sequencing using the Illumina NextSeq 550 platform essentially as described
elsewhere [17] (Benschop et al, manuscript submitted). In brief, cDNA was prepared from pre-
treated and extracted RNA from cell culture supernatant. Subsequently, double stranded DNA
was synthesized. Double stranded DNA was purified and concentrated and used to prepare
libraries using the Nextera XT DNA Library Prep Kit (Illumina). Sequencing was performed on
the NextSeq with the NextSeq 500/550 Mid Output Kit v2.5 (300 Cycles). Bioinformatic
analysis of obtained reads was performed using the Jovian pipeline with default parameters
(Schmitz et al, manuscript in prep; https://github.com/DennisSchmitz/Jovian).

Genetic and phylogenetic analysis

Mumps virus genotypes were determined based on SH sequences obtained from detected
mumps viruses by comparison with reference strains using BioNumerics version 7.6.3 [18–
20]. Genotyping was confirmed by basic local alignment search tool (BLAST) analysis [21, 22].

Obtained sequence data were aligned manually in MEGA7 and phylogenetic trees were
constructed using the maximum likelihood method with IQ-tree software via the webserver
(W-IQ-TREE) [23–25]. For each alignment, the best-fit substitution model according to bayes-
ian information criterion (BIC) was selected based on analysis with ModelFinder [26]. Branch
support in the different phylogenetic trees was calculated using the ultrafast bootstrap
approach (UFboot) with 1000 bootstrap alignments [27]. Phylogenetic trees were visualized
using FigTree v1.4.4 [28]. Complete and/or partial sequence data from mumps viruses mumps
virus genotype C (AY669145), Mumps virus Jeryl-Lynn (AF338106), Mumps virus strain
9218/Zg98 (EU370206), MuV/California,USA/50.07/1 (JX287386), MuV/DU.CR/O05
(EU370207), MuV/Iowa,USA/06/6 (JX287385), MuV/New York,USA/01.10 (JX287389),
MuV/New York,USA/40.09 (JX287390), MuV/New York,USA/53.09/3 (JX287387), MuVi.
Ontario.CAN/04.10 (KY006857), MuVi.Ontario.CAN/38.09/2 (KY680537), MuVi/Chennai.
IND/35.12 (KF843896), MuVi/Dadra&NagarHaveli.IND/42.16/2 (MG460606), MuVi/Davan-
gere.IND/01.14 (KX953297), MuVi/Gloucester.GBR/32.96 (AF280799), MuVi/Kushinagar.
IND/36.13 (KM385447), MuVi/London.GBR/03.02 (KF878082), MuVi/New Plymouth.NZL/
30.17 (MG765426), MuVi/Ontario.CAN/13.10 (KY006858), MuVi/Ontario.CAN/37.09
(KY006856), MuVi/Ontario.CAN/40.09/1 (KY680538), MuVi/Osmanabad.IND/10.12/17
(KF843894), MuVi/Osmanabad.IND/10.12/18 (KF843895), MuVi/Pune.IND/00.86
(KF738113), MuVi/Pune.IND/07.12 (KF738114), MuVi/Pune.IND/50.08 (KF843893), MuVi/
RW154.USA/0.70s (KF878080), MuVi/Split.CRO/05.11 (JN635498), MuVi/Springdale_754/
2016 (KY996512), MuVi/Zagreb.HRV/28.12 (KF481689), MuVs/Arkansas,USA/43.16/[G]
iso-late 20171006/BC06 (MK585579), MuVs/Illinois,USA./26.15/2 (MG986460), MuVs/Indiana.
USA/48.16 (MG986406), MuVs/Massachusetts,USA/21.16 (MF965260), MuVs/Massachu-
setts,USA/24.17/5 (MG986430), MuVs/Massachusetts,USA/26.15 (MF965222), MuVs/Massa-
setts,USA/30.16 (MF965299), MuVs/Massachusetts,USA/37.15 (MF965262), MuVs/
Massachusetts.USA/37.16 (MF965284), MuVs/Michigan.USA/4.16 (MG986419), MuVs/Montana.USA/11.16 (MG986420), MuVs/Ohio.USA/11.14 (MG986426), and MuVs/Pennsylvania.USA/19.16 (MG986458), were included in the alignments as reference sequences and to allow comparisons between mumps viruses detected in other countries and the Netherlands. Selection of these strains was based on BLAST analysis of obtained mumps virus near complete genomes and a manual selection of complete genomes of mumps genotype G viruses [11–13]. Pairwise identity analyses were performed in MEGA7 using the maximum likelihood method, complete deletion of missing data and otherwise default parameters [23].

Results

Mumps cases in 2017, 2018 and 2019 in the Netherlands

Between January 2017 and October 2019, 246 mumps cases were notified in the Netherlands based on clinical symptoms and laboratory confirmation, or an epidemiological link with a laboratory confirmed case. A mumps virus genotype could be determined for 123 cases (50%), of which 108 cases (88%) belonged to genotype G. The other 15 cases belonged to either genotype C (five cases), D (one case), H (seven cases), J (one case) or K (one case). From in total 82 mumps genotype G cases could NCRs sequence data be obtained in addition to the SH sequences used for genotyping (GenBank accession numbers MT238691-MT238955).

Virus isolation and next-generation sequencing

Inoculation of nine throat swabs and one oral fluid specimen (14%) resulted in a mumps virus isolate as determined by the presence of cytopathic effect. Eight mumps virus isolates belonged to genotype G, while the other two mumps virus isolates belonged to genotype C or K (Table 1). Near complete genomes (excluding 3' and 5' termini) were obtained from all isolates, with genome sizes ranging from 15144 to 15375 nucleotides (GenBank accession numbers MT238681-MT238690, European Nucleotide Archive numbers ERS4582384-90).

Comparisons between sequence data of original materials and isolates by Sanger sequencing

Comparison of nucleotide sequence data of SH+NCRs obtained from mumps viruses detected in clinical materials by Sanger sequencing with consensus sequence data from isolates obtained by NGS revealed in total three differences. At position 144 of the SH gene of MuVi/Deventer.NLD/12.19 a C was present (corresponding to position 6413 of the complete genome of Mumps virus -strain Jeryl-Lynn- live major vaccine component), while a T was detected at that position in the original material of MuVs/Deventer.NLD/12.19. Sanger sequencing confirmed the presence of a C at this position in the isolate. Additional Sanger sequencing revealed that the nucleotide shift from T to C was already present after the first passage.

At position 443 of the N-P NCR (corresponding to position 2141 of the complete genome of Mumps virus -strain Jeryl-Lynn- live major vaccine component), a C was detected in the sequence data from MuVi/Amsterdam.NLD/29.17/1, while an A was detected in the sequence data obtained from the original material (MuVs/Amsterdam.NLD/29.17/1). Since this nucleotide position was in the phosphoprotein gene, this nucleotide change resulted also in an amino acid change (lysine to glutamine). The presence of the C in the isolate was confirmed by Sanger sequencing. Additional Sanger sequencing analyses of the various passages indicated that the C was already detected at this position as a minor variant after the first passage, while two peaks of equal size were detected after the second passage. In the P-M NCR of MuVs/Utrecht.NLD/35.19, two peaks of similar size (A/G) were detected in the Sanger sequence data (both
(forward and reverse sequence) at position 76 (corresponding to position 2924 of the complete genome of Mumps virus -strain Jeryl-Lynn- live major vaccine component), while the consensus sequence of MuVi/Utrecht.NLD/35.19 was a G at that position. This nucleotide position was also in the phosphoprotein, but this nucleotide difference did not result in an amino acid change. Additional analysis of the first passage and second passage of this isolate by Sanger sequencing indicated that in all these passages both nucleotide variants were present with two peaks of equal size. No other nucleotide differences were detected between the sequence data obtained from the original materials and the isolates.

**Phylogenetic analysis of concatenated SH+NCRs sequences**

Phylogenetic analysis of concatenated SH+NCRs mumps genotype G virus sequences indicated that two main lineages of genotype G virus strains were detected in the Netherlands in 2017–2019 with respectively 49 and 17 mumps viruses (Fig 1), which differed by in total eight nucleotides in the SH and NCRs. Viruses of lineage I were only detected in the second half of 2018 and 2019, while viruses from lineage II were detected in 2017, 2018 and 2019. Another lineage of mumps genotype G viruses consisted of six cases, but no groups of viruses with identical sequence were detected within this lineage (lineage III). The presence of two main lineages was confirmed by analysis of near complete genomes of mumps viruses detected in these cases (Fig 2).

### Table 1. Overview of metadata of mumps virus isolates.

| Isolate            | Clinical specimen | Epidemiological link | Genotype | Mean read coverage | GenBank accession number | Genome size | Location\(^{a}\) of nucleotide differences between clinical material and isolate |
|--------------------|-------------------|----------------------|----------|--------------------|--------------------------|-------------|--------------------------------------------------------------------------------|
| MuVi/Amsterdam. NLD/29.17/1 | Throat swab     | Southern Europe, travelling. | G        | 3200               | MT238681                | 15361       | 2141A>C                                                                         |
| MuVi/Beugen. NLD/16.18 | Throat swab     | The Netherlands, unknown.    | G        | 4300               | MT238682                | 15284       | not detected                                                                   |
| MuVi/Eindhoven. NLD/42.18 | Oral fluid      | The Netherlands, cluster among students at a university. | G        | 550                | MT238684                | 15145       | not detected                                                                   |
| MuVi/Utrecht. NLD/8.19 | Throat swab     | Southeast Asia, travelling.  | K        | 2000               | MT238690                | 15363       | not detected                                                                   |
| MuVi/Noord-Holland. NLD/9.19 | Throat swab     | The Netherlands, unknown.    | G        | 2500               | MT238686                | 15365       | not detected                                                                   |
| MuVi/Deventer. NLD/12.19 | Throat swab     | The Netherlands, cluster among students at a university. | G        | 2300               | MT238683                | 15375       | 6413T>C                                                                         |
| MuVi/Utrecht. NLD/15.19 | Throat swab     | The Netherlands, contact with students at an university, possibly same cluster as MuVi/Deventer.NLD/12.19. | G        | 480                | MT238687                | 15375       | not detected                                                                   |
| MuVi/Utrecht. NLD/21.19 | Throat swab     | East-Asia, travelling.      | C        | 1400               | MT238689                | 15369       | not detected                                                                   |
| MuVi/Gelderland. NLD/26.19 | Throat swab     | The Netherlands, work.      | G        | 3700               | MT238685                | 15364       | not detected                                                                   |
| MuVi/Utrecht. NLD/35.19 | Throat swab     | The Netherlands, unknown.   | G        | 2600               | MT238688                | 15175       | 2924A/G>G                                                                     |

\(^{a}\) If the sequence consisted of two or more overlapping contigs by de-novo assembly, the mean coverage of the contig with the lowest coverage is indicated.

\#Location was based on the position in the complete genome of Mumps virus (strain Jeryl-Lynn) live major vaccine component (GenBank accession number AF338106).

[https://doi.org/10.1371/journal.pone.0233143.t001](https://doi.org/10.1371/journal.pone.0233143.t001)
Within these three lineages, multiple sequence variants were detected in the Netherlands. In addition, various other virus variants were detected in the Netherlands in 2017, 2018 and 2019, but these variants were only detected in a limited amount of cases. Based on epidemiological data, 69 mumps viruses included in this phylogenetic analysis were detected in cases that were infected in the Netherlands. From 9 cases, it was known that they were infected in another country, while the country of infection was unclear in 3 mumps cases. Mumps viruses
detected in 6 from these 9 cases that were not infected in the Netherlands belonged to lineage I, while the other three mumps viruses belonged to either lineage II (one mumps virus), lineage III (one mumps virus), or one of the viruses that were not part of a lineage (one mumps virus) (Fig 1).

**Comparison of genetic differences and phylogenetic topology between SH+NCRs and near complete genomes**

Comparison of the genetic differences provided by concatenated SH+NCRs sequences and near complete genomes revealed an increase in the number of nucleotide differences if near complete genomes were analyzed. For example, SH+NCRs sequences of MuVs/Utrecht.NLD/35.19 and MuVs/Eindhoven.NLD/42.18/1 were identical, while 11 nucleotide differences were detected by analysis of near complete genomes. The pairwise identities for these two viruses were 100% for the SH+NCRs and 99.97% for the near complete genomes. In addition, one nucleotide difference was present between the SH+NCRs sequences of MuVs/Amsterdam.NLD/29.17/1 and MuVs/Beugen.NLD/16.18, while 16 nucleotide differences were present between the isolated near complete genomes of these two viruses, although one of these differences was confirmed to be related to isolation of the virus. Pairwise identities between the isolates of these viruses were 99.91% for the SH+NCRs and 99.87% for the near complete genomes. Furthermore, MuVs/Deventer.NLD/12.19 and MuVs/Utrecht.NLD/15.19 were identical for SH+NCRs sequences, while four nucleotide differences were present between the near complete genomes of these isolated viruses (including one related to isolation of the virus), resulting in pairwise identities of 99.96% for the SH+NCRs and 99.97% for the near complete genomes (Fig 2). The average pairwise identity between the eight mumps virus isolates was 99.60% for the SH+NCRs and 99.66% for the near complete genomes.

Comparison of the phylogenetic topology between SH+NCRs and near complete genomes indicated that a similar branching pattern was observed in both phylogenetic trees, except for the branching pattern of MuVi/Utrecht.NLD/35.19 and MuVi/Gelderland.NLD/26.19/2. The two nucleotide changes in SH+NCRs of MuVi/Deventer.NLD/12.19 and MuVi/Amsterdam.NLD/29.17/1 compared to clinical samples resulted in increased branch lengths, but did not change the phylogenetic topology (Fig 2).

**Phylogenetic analysis of near complete mumps viruses**

Alignment and subsequent phylogenetic analysis of the near complete genomes (excluding 3’ and 5’ termini) obtained from mumps virus isolates detected in the Netherlands with several representative and closely related mumps viruses indicated that these viruses also belonged to two separate groups of viruses (Fig 3). Pairwise identity analysis on the nucleotide level of mumps genotype G viruses of the near complete genomes of MuVi/Gelderland.NLD/26.19/2, MuVi/Utrecht.NLD/35.19, MuVi/Eindhoven.NLD/42.18 and MuVi/Noord-Holland.NLD/9.19 confirmed that these viruses were most closely related to MuVs/Michigan.USA/4.16 (99.90–99.91%) and MuVs/Massachusetts.USA/21.16 (99.56–99.88%). MuVi/Amsterdam.NLD/29.17/1, MuVi/Beugen.NLD/16.18, MuVi/Deventer.NLD/12.19 and MuVi/Utrecht.NLD/15.19 were most closely related to MuVs/Montana.USA/11.16 and MuVs/Illinois.USA/26.15/2 (99.59–99.63%). Mumps virus genotype K virus MuVi/Utrecht.NLD/8.19 was most closely related to MuVs/Massachusetts.USA/24.17/5[K] (99.37%) and mumps virus genotype C virus MuVi/Utrecht.NLD/21.19 was most closely related to MuVi/Kushinagar.IND/36/13[C] (99.01%) (Fig 3).
Discussion

Mumps cases continue to occur, also in countries with a relatively high vaccination rate. Molecular epidemiology could contribute to the understanding of mumps virus circulation. In
the present study, the molecular epidemiology of mumps virus was evaluated using SH+NCRs mumps virus sequences obtained from mumps cases in the Netherlands in 2017–2019. Analysis of these regions with the relatively highest variation of the mumps virus genome revealed the presence of two major lineages in the Netherlands. The presence of these two lineages was confirmed by analysis of near complete genomes of a selection of mumps viruses.

The presence of two lineages with multiple closely related mumps viruses in recent years, might indicate that there was (intermittent) circulation of mumps viruses in the Netherlands in recent years. This is supported by the fact that near complete genomes of both lineages are distinct from mumps viruses detected recently in mainly the United States of America and Canada [11, 29]. Of interest, viruses of lineage I were not detected before the second part of 2018, suggesting that this lineage emerged recently in the Netherlands. However, recent SH+NCRs mumps virus sequences from nearby countries were not available and therefore it is not possible to draw conclusions. In addition to mumps genotype G viruses, mumps viruses were detected with other mumps genotypes. Since these mumps viruses were detected only in a relatively small proportion, these viruses were most likely not endemic in the Netherlands in recent years.

Of interest, comparison of SH+NCRs sequence data from original materials with that of isolates indicated that passaging of mumps viruses over cells resulted in three nucleotide differences. Since the sequence of 2270 nucleotides of in 10 total viruses were compared, it was calculated that 0.013% of all nucleotide positions changed due to passaging (15384*0.00013 = ~2 nucleotide changes for a full mumps virus genome). Therefore, sequence data from isolates should be interpreted carefully, especially if used to understand exact transmission trees. To study transmission trees, preferably complete genomes obtained from original materials or eventually viruses that are passaged only a single time over cells are used [11, 29].

Most mumps viruses from which a near complete genome was obtained in this study were collected from mumps cases that occurred several months after each other, and therefore it cannot be concluded whether these viruses are part of the same transmission chain. Of interest, mumps viruses MuVi/Noord-Holland.NLD/9.19, MuVi/Deventer.NLD/12.19 and MuVi/Utrecht.NLD/15.19 were collected within six weeks. While the sequence of MuVi/Noord-Holland.NLD/9.19 was relatively distinct from the other two viruses, between MuVi/Utrecht.NLD/15.19 and MuVi/Deventer.NLD/12.19 were only four nucleotide differences present. Since both mumps cases also lived in the same city, they were most likely part of the same mumps cluster, but there was no direct epidemiological link between these two cases.

Comparison of phylogenetic trees prepared by analysis of SH+NCRs and near complete genomes indicated that the topologies of both trees were similar, while branches lengths were different. Furthermore, the mean pairwise identity of the SH+NCRs regions was slightly lower than the mean pairwise identity of the near complete genomes (99.60% vs. 99.66%), which confirms that the SH+NCRs regions are relatively variable regions of the mumps virus genome [18]. Therefore, analysis of SH+NCRs sequences is a useful approach for molecular surveillance [7, 8]. However, to study exact transmissions trees, preferably complete genomes are analyzed [11, 29].

In conclusion, while in recent years no major outbreaks of mumps occurred in the Netherlands, the presence of two main lineages of multiple mumps genotype G viruses with identical or very similar SH+NCRs sequences might indicate (intermittent) circulation of mumps viruses in the Netherlands in 2017–2019.

**Acknowledgments**

The authors wish to thank medical microbiological laboratories and municipal health services for collecting and submitting clinical specimens that are used in this study and Tim Severs and Harry Vennema for setting-up NGS protocols.
Author Contributions

Conceptualization: Rogier Bodewes.

Data curation: Linda Reijnen, Jeroen Kerkhof, Jeroen Cremer, Dennis Schmitz, Irene K. Veldhuijzen.

Formal analysis: Rogier Bodewes, Linda Reijnen, Jeroen Kerkhof, Jeroen Cremer, Rob van Binnendijk, Irene K. Veldhuijzen.

Funding acquisition: Linda Reijnen.

Investigation: Rogier Bodewes, Jeroen Kerkhof, Jeroen Cremer.

Methodology: Linda Reijnen, Dennis Schmitz.

Project administration: Rogier Bodewes.

Software: Dennis Schmitz.

Writing – original draft: Rogier Bodewes.

Writing – review & editing: Rob van Binnendijk, Irene K. Veldhuijzen.

References

1. Philip RN, Reinhard KR, Lackman DB. Observations on a mumps epidemic in a “virgin” population. 1958. Am J Epidemiol. 1995; 142(3):233–53; discussion 1–2. PMID: 7631629.

2. Zamir CS, Schroeder H, Shooib H, Abramson N, Zentner G. Characteristics of a large mumps outbreak: Clinical severity, complications and association with vaccination status of mumps outbreak cases. Hum Vaccin Immunother. 2015; 11(6):1413–7. https://doi.org/10.1080/21645515.2015.1021522 PMID: 25874726; PubMed Central PMCID: PMC4514179.

3. Dittrich S, Hahne S, van Lier A, Kohl R, Boot H, Koopmans M, et al. Assessment of serological evidence for mumps virus infection in vaccinated children. Vaccine. 2011; 29(49):9271–5. Epub 2011/10/11. https://doi.org/10.1016/j.vaccine.2011.09.072 PMID: 21983156.

4. Hirasing RA, Schaapveld K. [Vaccination against mumps successful]. Ned Tijdschr Geneeskd. 1993; 137(30):1498–500. PMID: 8366936.

5. Sane J, Gouma S, Koopmans M, de Melker H, Swaan C, van Binnendijk R, et al. Epidemic of mumps among vaccinated persons, The Netherlands, 2009–2012. Emerg Infect Dis. 2014; 20(4):643–8. https://doi.org/10.3201/eid2004.131681 PMID: 24655811; PubMed Central PMCID: PMC3966393.

6. National Institute for Public Health and the Environment (RIVM). The National Immunisation Programme in the Netherlands: Surveillance and developments in 2018–2019. Schurink-van ‘t Klooster TM, De Melker H, editors. 2019.

7. Gavilan AM, Fernandez-Garcia A, Rueda A, Castellanos A, Masa-Calles J, Lopez-Perea N, et al. Genomic non-coding regions reveal hidden patterns of mumps virus circulation in Spain, 2005 to 2015. Euro Surveill. 2018; 23(15). https://doi.org/10.2807/1560-7917.ES.2018.23.15.00349 PMID: 29667574.

8. Bodewes R, van Roonen K, Cremer J, Veldhuijzen IK, van Binnendijk R. Optimizing molecular surveillance of mumps genotype G viruses. Infect Genet Evol. 2019; 69:230–4. Epub 2019/02/11. https://doi.org/10.1016/j.meegid.2019.02.005 PMID: 30738791.

9. Gouma S, Cremer J, Parkkali S, Veldhuijzen I, van Binnendijk RS, Koopmans MPG. Mumps virus F gene and HN gene sequencing as a molecular tool to study mumps virus transmission. Infect Genet Evol. 2016; 45:145–50. https://doi.org/10.1016/j.meegid.2016.08.033 PMID: 27590714.

10. Barrabeig I, Anton A, Torner N, Pumarola T, Costa J, Dominguez A, et al. Mumps: MMR vaccination and genetic diversity of mumps virus, 2007–2011 in Catalonia, Spain. BMC Infect Dis. 2019; 19(1):954. Epub 2019/11/11. https://doi.org/10.1186/s12879-019-4496-z PMID: 31706275; PubMed Central PMCID: PMC6842476.

11. Stapleton PJ, Eshaghii A, Seo CY, Wilson S, Harris T, Deeks SL, et al. Evaluating the use of whole genome sequencing for the investigation of a large mumps outbreak in Ontario, Canada. Sci Rep. 2019; 9(1):12615. Epub 2019/09/01. https://doi.org/10.1038/s41598-019-47740-1 PMID: 31471545; PubMed Central PMCID: PMC6717193.

12. Moncla L, and the Nextstrain project team. Real-time tracking of mumps virus evolution. Available from: https://nextstrain.org/mumps/global. Site accessed April 21, 2020.
13. Alkam D, Jenjaroenpun P, Wongsurawat T, Udaondo Z, Patumcharoenpol P, Robeson M, et al. Genomic characterization of mumps viruses from a large-scale mumps outbreak in Arkansas, 2016. Infect Genet Evol. 2019; 75:103965. Epub 2019/07/19. https://doi.org/10.1016/j.meegid.2019.103965 PMID: 31319177; PubMed Central PMCID: PMC6832845.

14. Hadfield J, Megill C, Bell SM, Huddleston J, Potter B, Callender C, et al. Nextstrain: real-time tracking of pathogen evolution. Bioinformatics. 2018; 34(23):4121–3. Epub 2018/05/24. https://doi.org/10.1093/bioinformatics/bty407 PMID: 29790939; PubMed Central PMCID: PMC6247931.

15. Sagulenko P, Puller V, Neher RA. TreeTime: Maximum-likelihood phylogenetic analysis. Virus Evol. 2018; 4(1):ve0042. Epub 2018/01/18. https://doi.org/10.1093/ve/ve0042 PMID: 29340210; PubMed Central PMCID: PMC5758920.

16. Uchida K, Shinhara M, Shimada S, Segawa Y, Doi R, Gotoh A, et al. Rapid and sensitive detection of mumps virus RNA directly from clinical samples by real-time PCR. J Med Virol. 2005; 75(3):470–4. Epub 2005/01/14. https://doi.org/10.1002/jmv.20291 PMID: 15648065.

17. Hasan MR, Rawat A, Tang P, Jithesh PV, Thomas E, Tan R, et al. Depletion of Human DNA in Spiked Clinical Specimens for Improvement of Sensitivity of Pathogen Detection by Next-Generation Sequencing. J Clin Microbiol. 2016; 54(4):919–27. Epub 2016/01/15. https://doi.org/10.1128/JCM.03050-15 PMID: 26763966; PubMed Central PMCID: PMC4807931.

18. Jin L, Orvell C, Myers R, Rota PA, Nakayama T, Forcic D, et al. Genomic diversity of mumps virus and global distribution of the 12 genotypes. Rev Med Virol. 2015; 25(2):85–101. https://doi.org/10.1002/rmv.1819 PMID: 25424978.

19. World Health Organization (WHO). Mumps virus nomenclature update, 2012. Weekly Epidemiological Record (WER). 2012; 87[22]:217–24.

20. Jin L, Rima B, Brown D, Orvell C, Tecle T, Afzal M, et al. Proposal for genetic characterisation of wild-type mumps strains: preliminary standardisation of the nomenclature. Arch Virol. 2005; 150(9):1903–9. Epub 2005/06/17. https://doi.org/10.1007/s00705-005-0563-4 PMID: 15959834.

21. Morgulis A, Coulouris G, Raytsev Y, Madden TL, Agarwala R, Schaffer AA. Database indexing for production MegaBLAST searches. Bioinformatics. 2008; 24(16):1757–64. Epub 2008/06/24. https://doi.org/10.1093/bioinformatics/btn322 PMID: 18567917; PubMed Central PMCID: PMC2696921.

22. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol. 1990; 215(3):403–10. Epub 1990/10/05. https://doi.org/10.1016/S0022-2836(05)80360-2 PMID: 2231712.

23. Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Mol Biol Evol. 2016; 33(7):1870–4. Epub 2016/03/24. https://doi.org/10.1093/molbev/msw054 PMID: 27004904.

24. Nguyen LT, Schmidt HA, von Haeseler A, Minh BO. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol. 2015; 32(1):268–74. Epub 2014/11/06. https://doi.org/10.1093/molbev/msu210 PMID: 25371430; PubMed Central PMCID: PMC4271533.

25. Trifinopoulos J, Nguyen LT, von Haeseler A, Minh BO. W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. Nucleic Acids Res. 2016; 44(W1):W232–5. Epub 2016/04/17. https://doi.org/10.1093/nar/gkw1256 PMID: 27084950; PubMed Central PMCID: PMC4987875.

26. Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jerumiin LS. ModelFinder: fast model selection for accurate phylogenetic estimates. Nat Methods. 2017; 14(6):587–9. Epub 2017/05/10. https://doi.org/10.1038/nmeth.4285 PMID: 28481363; PubMed Central PMCID: PMC5453245.

27. Minh BQ, Nguyen MA, von Haeseler A. Ultrafast approximation for phylogenetic bootstrap. Mol Biol Evol. 2013; 30(5):1188–95. Epub 2013/02/19. https://doi.org/10.1093/molbev/ms3042 PMID: 23418397; PubMed Central PMCID: PMC3670741.

28. Rambaut A. FigTree v1.4.4. 2018.

29. Wohl S, Metsky HC, Schaffner SF, Piantadosi A, Burns M, Lewnard JA, et al. Combining genomics and epidemiology to track mumps virus transmission in the United States. PLoS Biol. 2020; 18(2):e3000611. Epub 2020/02/12. https://doi.org/10.1371/journal.pbio.3000611 PMID: 32045407; PubMed Central PMCID: PMC7012397.