Infection, Genetics and Evolution 69 (2019) 230–234

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Short communication

Optimizing molecular surveillance of mumps genotype G viruses

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ARTICLE INFO

Keywords:
Mumps virus
Molecular surveillance
Sequencing
Genotype G
Molecular epidemiology
Mumps

ABSTRACT

Mumps viruses continue to cause sporadic cases and outbreaks in countries with a high vaccination coverage for mumps. Molecular surveillance of mumps viruses can be supportive to elucidate the origin and transmission routes of mumps virus in case of an outbreak. Currently, molecular surveillance is worldwide primarily focused on sequencing of the small hydrophobic (SH) gene. However, few studies have already shown that additional genes or regions contribute to the resolution of the sequence data in such a way that mumps cases that seem to be linked to the same source on basis of the SH sequence, appear to be linked to another source or chain of transmission. Notably, this sequence information was recently extracted from the hemagglutinin-neuraminidase (HN) and fusion (F) genes (total 3364 nucleotides), or from the sum of the three non-coding regions (NCRs; total 1954 nt) between the nucleocapsid protein, phosphoprotein, matrix protein and F protein, but also from the complete genome. Here, sequence data from NCRs were compared with that of the HN and F gene, using mumps genotype G viruses detected in the Netherlands between 2010 and 2018. Results of this study indicate that NCRs sequence data provided similar or slightly better sequence resolution compared to the HN and F genes for most viruses. For molecular surveillance of currently circulating mumps genotype G viruses is sequencing of SH in combination with NCRs currently a useful approach.

1. Main text

Mumps is an acute illness characterized by a temporary unilateral or bilateral swelling of the parotid glands caused by infection with mumps virus (Hviid et al., 2008). In addition, infection with mumps virus can result in various complications (Philip et al., 1995; Zamir et al., 2015). However, infection with mumps virus does not result in recognized clinical signs in about a third of unvaccinated persons, and in an estimated three-quarters of vaccinated persons (Dittrich et al., 2011; Philip et al., 1995).

Vaccination against mumps virus was implemented in the National Immunization Program in the Netherlands in 1987, which resulted in a rapid decline of mumps cases (Hirasing and Schaapveld, 1993). However, in recent years various outbreaks occurred in the Netherlands and various other countries mainly among vaccinated young adults. The last major outbreaks with over 1500 reported cases in the Netherlands lasted for three seasons, from 2009 to 2012 (Sane et al., 2014). From 2013 onwards, only relatively small local outbreaks and sporadic mumps cases were reported.

Especially when the number of cases is limited, it is of interest to understand whether reported cases are the result of endemic circulation of mumps virus in a certain area, or introduction of a new and genetically distinct mumps virus. The use of sequencing data could provide support in understanding the epidemiology of mumps viruses.

Although sequencing of mumps viruses is currently still primarily focused on sequencing of the SH gene and adjacent non-coding regions (SH region; 316 nucleotides), various recent studies have highlighted the importance of obtaining additional sequence information (WHO, 2012; Gavilan et al., 2018; Gouma et al., 2016a; Jin et al., 2015). While in one study sequencing of hemagglutinin-neuraminidase protein gene (HN) and fusion protein gene (F) was reported, another study described sequencing of the non-coding regions (NCRs) located between nucleocapsid protein and phosphoprotein (N-P), phosphoprotein and matrix protein (P-M), and matrix protein gene and F gene (M-F) (Gavilan et al., 2018; Gouma et al., 2016a).

These studies collectively showed that sequencing of additional

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https://doi.org/10.1016/j.meegid.2019.02.005
Received 17 December 2018; Received in revised form 4 February 2019; Accepted 6 February 2019
Available online 07 February 2019

parts of the genome contribute to the resolution of the sequence data in such a way that mumps cases that might belong to the same molecular cluster on basis of the SH sequence, can be distinguished in separate molecular clusters, indicating multiple transmission chains from different sources. Obtaining the complete genome could result in the most complete data, but this is currently often hampered by the relative high costs of next-generation sequencing. In addition, at the moment next-generation sequencing does also often not provide an accurate and complete sequence from low amount of mumps virus RNA in clinical samples without pre-amplification, especially in vaccinated cases (Gouma et al., 2016b). Therefore, the aim of the present study was to elucidate to what extent Sanger sequencing of either NCRs or HN and F gene provided sufficient information for the molecular surveillance of mumps genotype G viruses in the Netherlands.

To this end, mumps viruses that were obtained from clinical cases notified under the Public Health Act in the Netherlands were subjected to sequencing of the SH region, HN and F gene and NCRs essentially as described previously (Gavilan et al., 2018; Gouma et al., 2014, 2016a). Mumps virus sequence data were all generated from mumps virus RNA extracted from oral fluid or throat swabs, except for MuVs/Amsterdam.NLD/29.17/2 which was obtained from urine. No sequence differences were observed in the consensus sequence of mumps virus RNA detected in urine, oral fluid or throat swabs from the same patients during a pilot study (data not shown). All sequence data of SH, HN and F genes from mumps viruses detected between 2010 and 2015 were obtained from patients described in a previous study (Gouma et al., 2016a). NCRs sequence data from mumps viruses detected in 2010–2018 and SH, HN and F genes from viruses detected in 2016–2018 were obtained in the current study. Obtained sequence data is available on Genbank, accession numbers MK276692-MK276877. Phylogenetic analysis of obtained sequence data was performed in MEGA7 using maximum-likelihood method with the Hasegawa-Kishino-Yano model and 500 bootstrap replicates (Kumar et al., 2018). For analysis, multiple parts of the genome of mumps viruses were taken together by concatenating these different parts.

First, sequence data of NCRs were compared to sequence data of SH, HN and F genes of mumps genotype G viruses (Gouma et al., 2016a). To this end, NCRs sequences (total 1954 nucleotides) were determined from a selection of mumps viruses with highly similar SH, F and HN gene sequences reported in 2010 and 2011 (group A; 10 viruses) to elucidate if NCRs sequences could provide similar or additional resolution. Within these 10 viruses, at 7 positions nucleotide differences were present in four viruses. In addition, NCRs were obtained from a selection of mumps viruses detected in 2013–2015 (groups B; 6 viruses and C; 4 viruses; Fig. 1, Fig. S1). Viruses were selected based on HN, F and SH sequence similarity, representativeness of different epidemiological clusters and availability of clinical material. Though mumps viruses of groups B and C were identical on basis of SH gene sequencing, they could be distinguished by sequence analysis of both the NCRs and HN and F genes (Fig. 1). In addition, additional sequence variation was detected in the NCRs of mumps viruses of group A. Here, MuVs/Leeuwarden.NLD/49.10 and MuV/Aerdenhout.NLD/35.10 showed one and two nucleotide differences in the NCRs, respectively when compared to other mumps viruses, while two others (MuVs/Bussum.NLD/46.11 and MuVs/Bussum.NLD/49.11) separated completely from group A on basis of two nucleotide differences. Nucleotide differences detected in these four viruses were detected in three different NCRs. Therefore, it was concluded that sequence data of NCRs could distinguish similar groups as sequence data of HN and F genes, and provided some additional within-group sequence variation.

Subsequently, more recently notified mumps cases in the Netherlands were analyzed. From 136 laboratory confirmed mumps cases notified between January 2016 and August 2018, the SH gene sequences were obtained from 56 cases, of which 42 (75%) were mumps virus genotype G. From these detected mumps genotype G viruses, complete SH, F, HN and NCRs sequence data could be obtained from 21 mumps viruses, representing multiple clusters and individual cases. Analysis of SH regions of these mumps viruses indicated that 8 mumps viruses were identical with the group B and C mumps viruses from before and those detected in other countries, while 7 mumps viruses were identical with mumps viruses detected in other countries only. In addition, sequences of SH regions of 6 mumps viruses were different from currently known mumps viruses (Fig. S2).

Analysis of NCRs and SH sequence data of these recent mumps genotype G viruses provided additional sequence variation compared to analysis of SH, a part of these viruses could also be epidemiologically linked based on temporal and geographic data (Fig. 2). For example, two clusters of mumps cases, from Valkenswaard and Tilburg could be distinguished by analyzing NCRs sequence data.

Comparison of sequence data of the SH and NCRs with F, HN and SH indicated that information provided by analysis of genetic data of most viruses was similar. For four viruses, some additional genetic variation was detected in NCRs compared to closely related viruses, which was not present in F and HN genes. Vice versa, F and HN may also add some additional genetic information (MuVs/Valkenswaard/11.16/1; Fig. 2).

Of interest, analysis of NCRs sequence data of mumps genotype G viruses in the Netherlands and Spain also demonstrated that a number of mumps viruses detected in both countries were identical or had highly similar NCRs sequences (Fig. S3). However, no known epidemiological link existed between the Spanish and Dutch sequences. In addition, NCRs sequence data was only available from a small proportion of mumps viruses detected in these countries and for a very limited number of viruses from the same year. Therefore, additional data is needed to understand the circulation of mumps genotype G viruses within certain parts of Europe.

In conclusion, results of this study confirmed that sequencing of the NCRs in combination with SH provided useful information for molecular surveillance of mumps genotype G viruses (Gavilan et al., 2018). Although most complete information is provided by obtaining full genomes, whether this will significantly increase the sequence resolution of mumps viruses needs to be determined. In conclusion, molecular surveillance of mumps genotype G viruses using NCRs and SH sequence data is currently a useful, relatively low-cost, alternative.

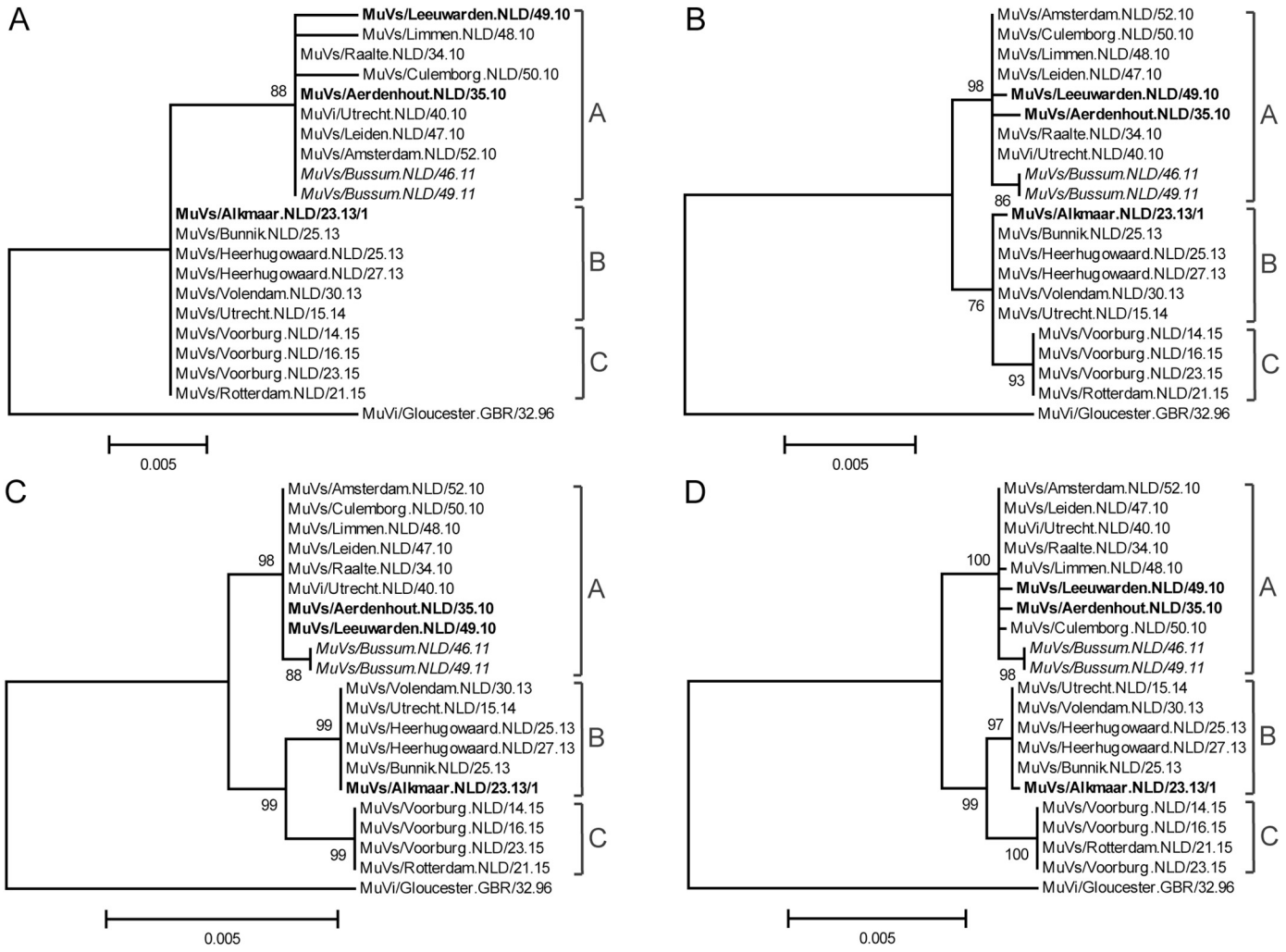

Fig. 1. Comparison of different genomic regions for molecular surveillance of selected mumps viruses in 2010–2015. Phylogenetic analysis was performed of the SH gene and adjacent NCRs (A), concatenated sequences of the N-P, P-M and M-F NCRs (B) and the F and HN (C) gene and all genomic regions combined (D) of mumps genotype G viruses detected in the Netherlands in 2010–2015 with the HKY model and 500 bootstrap replicates. For this analysis, three groups of mumps viruses with an identical or similar SH sequence were selected (Gouma et al., 2016a). Only bootstrap values > 70 are indicated. Viruses with additional sequence variation in the NCRs compared to F and HN are indicated in bold, while viruses with additional sequence variation in both the NCRs and HN and F are in italic.

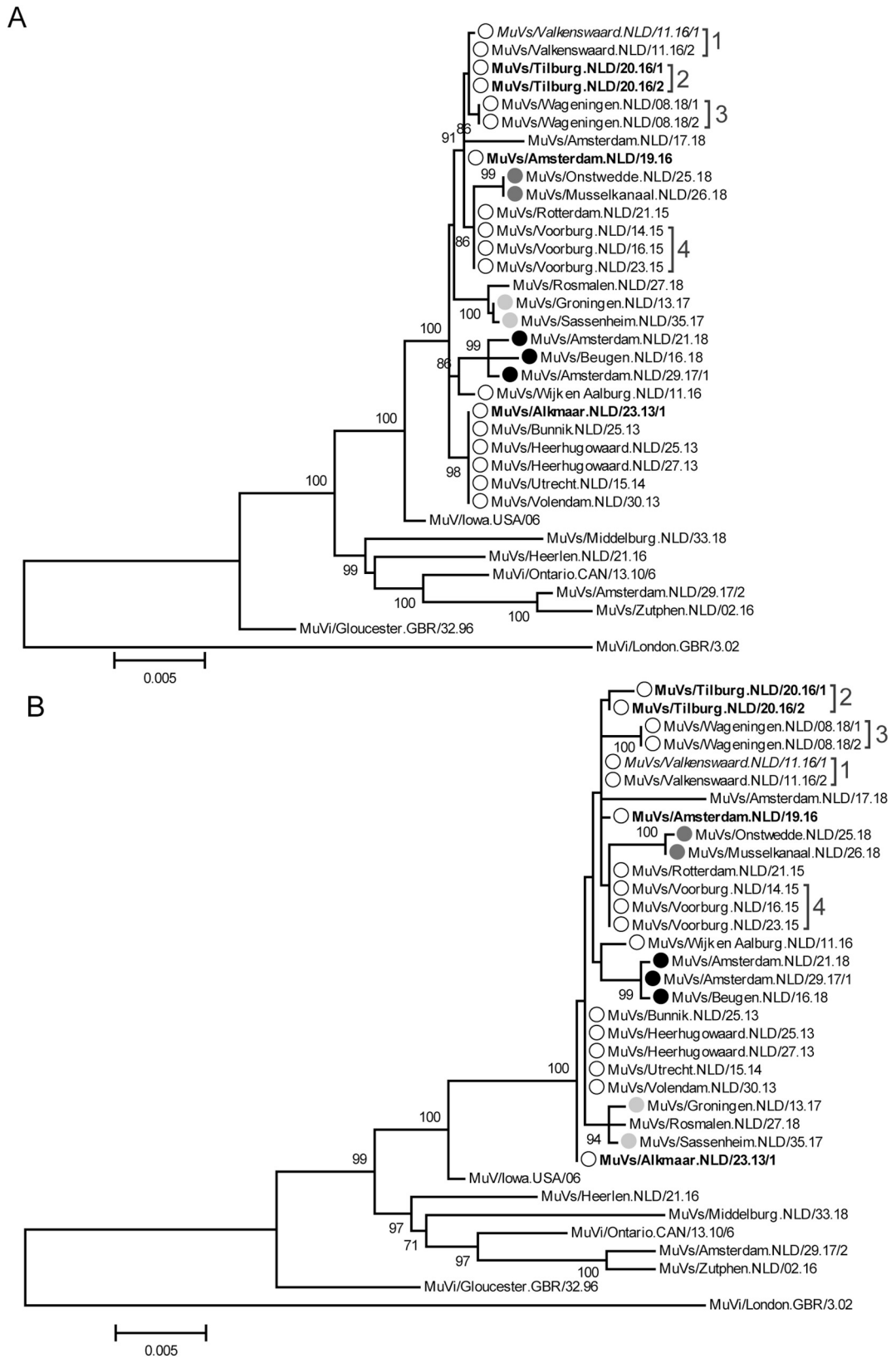

Fig. 2. Molecular surveillance of recent mumps genotype G viruses in the Netherlands. Phylogenetic analysis was performed on the concatenated sequences of the SH, HN and F gene (A), and NCRs and SH gene (B) of mumps genotype G viruses detected in the Netherlands in 2013–2018 with the HKY model and 500 bootstrap replicates. Only bootstrap values > 70 are indicated. Groups of viruses with identical SH sequences are indicated with white, light grey, dark grey and black circles. Viruses with additional sequence variation in the NCR compared to the F and H genes are indicated with names in bold, while the virus with additional variation in the F and HN genes are indicated in italic. Viruses belonging to the same epidemiological cluster are indicated with numbers.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2019.02.005.

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