



Complete Genome Sequences of Six Measles Virus Strains

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ABSTRACT Genetic characterization of wild-type measles virus (MV) strains is a critical component of measles surveillance and molecular epidemiology. We have obtained complete genome sequences of six MV strains belonging to different genotypes, using random-primed next generation sequencing.

Measles is one of the most contagious human diseases and is still responsible for considerable childhood morbidity and mortality. The causative agent, measles virus (MV), is an enveloped virus with a negative-sense single-stranded RNA genome and is a member of the genus *Morbillivirus* within the family *Paramyxoviridae* (1). MV genomes are relatively stable; the virus consists of a single serotype, and live-attenuated MV vaccines developed in the 1960s still confer protection against currently circulating wild-type MV strains. However, the MV genome also contains a number of variable regions that have been used to assign 8 genetic clades (A to H), which have been further subdivided into 24 genotypes (or subtypes) (2). The MV genome is typically 15,894 nucleotides (nt) in length, and it encodes 6 structural proteins (N, P, M, F, H, and L) and two nonstructural proteins (V and C).

Viral isolates of circulating wild-type MV strains provide an important resource for virology and vaccine development. In this work, we have obtained complete genome sequences for the following six wild-type MV strains: MVi/Khartoum.SUD/34.97/2 [B3], a strain endemically circulating in Khartoum (Sudan) in 1997 (3); MVi/Bilthoven.NLD/1991 [C2], a strain isolated during a measles outbreak in The Netherlands in 1991 (4); MVi/Amsterdam.NLD/19.11 [D4], an unpublished import case into The Netherlands isolated in 2011 from a patient who had traveled to Greece; MVi/Dodewaard.NLD/29.13 [D8], an isolate obtained during a large measles outbreak in The Netherlands in 2013 (5); MVi/Amsterdam.NLD/49.97 [G2], an isolate obtained from a secondary case in a hospital outbreak in 1997 (6); and MVi/Amsterdam.NLD/27.97, a virus isolated from a measles patient in 1997 with a recent history of travel to China (7). All viruses were isolated in Epstein-Barr virus-transformed human B-lymphoblastic cell lines, followed by short-term culture (maximum 3 passages) in Vero cells expressing human CD150 (8). All cultures were confirmed negative for *Mycoplasma* spp. All virus isolates are available via the European Virus Archive (<https://www.european-virus-archive.com>).

Total viral nucleic acid was extracted from 6 virus cultures (with titers between 10^5 and 10^7 50% tissue culture infective dose [TCID₅₀]/ml), using Bioke extraction reagents (Leiden, The Netherlands). Extracted RNA was reverse transcribed and second-strand synthesis was performed using random primers as previously described (9), followed by sequencing on the Ion Torrent S5XL platform to generate 2.2×10^6 to 2.8×10^6 400-nt reads per sample. Raw reads were trimmed from the 3' end to a median Phred score of 30 and minimum length of 75 nt using the Quality Assessment of Short Read (QUASR) package (10), and then *de novo* assembled using SPAdes version 3.11 (11).

Six complete MV genomes were obtained, including the first full genome for MV genotype C2. Of note, one MV genome (MVi/Amsterdam.NLD/19.11 [D4]) had a

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6-nucleotide insertion, giving rise to a full genome length of 15,900 nt and complying with the rule of six for replication competency in MV (12). These sequence data will provide a useful reference for measles surveillance and for studies to better understand MV evolution and biology.

Accession number(s). The MV sequences described in this study have been deposited in GenBank under the accession numbers [MG912589](#) to [MG912594](#).

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REFERENCES

1. Rota PA, Moss WJ, Takeda M, De Swart RL, Thompson KM, Goodson JL. 2016. Measles. *Nat Rev Dis Primers* 2:16049. <https://doi.org/10.1038/nrdp.2016.49>.
2. World Health Organization. 2015. Genetic diversity of wildtype measles viruses and the global measles nucleotide surveillance database (MeaNS). *Wkly Epidemiol Rec* 30:373–380.
3. El Mubarak HS, van de Bildt MWG, Mustafa OA, Vos HW, Mukhtar MM, Ibrahim SA, Andeweg AC, El Hassan AM, Osterhaus ADME, de Swart RL. 2002. Genetic characterization of wild-type measles viruses circulating in suburban Khartoum, 1997–2000. *J Gen Virol* 83:1437–1443. <https://doi.org/10.1099/0022-1317-83-6-1437>.
4. van Binnendijk RS, van der Heijden RWJ, van Amerongen G, UytdeHaag FGCM, Osterhaus ADME. 1994. Viral replication and development of specific immunity in macaques after infection with different measles virus strains. *J Infect Dis* 170:443–448. <https://doi.org/10.1093/infdis/170.2.443>.
5. Woudenberg T, van Binnendijk RS, Sanders EAM, Wallinga J, de Melker HE, Ruijs WLM, Hahné SJM. 2017. Large measles epidemic in The Netherlands, May 2013 to March 2014: changing epidemiology. *Euro Surveill* 22. <https://doi.org/10.2807/1560-7917.ES.2017.22.3.30443>.
6. de Swart RL, Wertheim-van Dillen PME, van Binnendijk RS, Muller CP, Frenkel J, Osterhaus ADME. 2000. Measles in a Dutch hospital introduced by an immunocompromised infant from Indonesia infected with a new virus genotype. *Lancet* 355:201–202. [https://doi.org/10.1016/S0140-6736\(99\)04652-8](https://doi.org/10.1016/S0140-6736(99)04652-8).
7. Truong AT, Mulders MN, Gautam DC, Ammerlaan W, de Swart RL, King CC, Osterhaus ADME, Muller CP. 2001. Genetic analysis of Asian measles virus strains—new endemic genotype in Nepal. *Virus Res* 76:71–78. [https://doi.org/10.1016/S0168-1702\(01\)00255-6](https://doi.org/10.1016/S0168-1702(01)00255-6).
8. Ono N, Tatsuo H, Hidaka Y, Aoki T, Minagawa H, Yanagi Y. 2001. Measles viruses on throat swabs from measles patients use signaling lymphocytic activation molecule (CDw150) but not CD46 as a cellular receptor. *J Virol* 75:4399–4401. <https://doi.org/10.1128/JVI.75.9.4399-4401.2001>.
9. Phan MVT, Anh PH, Van Cuong N, Oude Munnink BB, van der Hoek L, Tri TN, Bryant JE, Baker S, Thwaites G, Woolhouse M, Kellam P, Rabaa MA, Cotten M. 2016. Unbiased whole-genome deep sequencing of human and porcine stool samples reveals circulation of multiple groups of rotaviruses and a putative zoonotic infection. *Virus Evol* 2:58875. <https://doi.org/10.1093/ve/vew027>.
10. Watson SJ, Welkers MRA, Depledge DP, Coulter E, Breuer JM, de Jong MD, Kellam P. 2013. Viral population analysis and minority-variant detection using short read next-generation sequencing. *Philos Trans R Soc Lond B Biol Sci* 368:20120205. <https://doi.org/10.1098/rstb.2012.0205>.
11. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Pribelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 19:455–477. <https://doi.org/10.1089/cmb.2012.0021>.
12. Bankamp B, Liu C, Rivaviller P, Bera J, Shrivastava S, Kirkness EF, Bellini WJ, Rota PA. 2014. Wild-type measles viruses with non-standard genome lengths. *PLoS One* 9:e95470. <https://doi.org/10.1371/journal.pone.0095470>.