



HAL
open science

Epidemiological and genetic characterization of measles virus circulating strains at Marseille, France during 2017–2019 measles outbreak

Rita Jaafar, Christine Zandotti, Clio Grimaldier, Maëlia Etoundi, Ines Kadri, Celine Boschi, Priscilla Jardot, Philippe Colson, Didier Raoult, Bernard La Scola, et al.

► To cite this version:

Rita Jaafar, Christine Zandotti, Clio Grimaldier, Maëlia Etoundi, Ines Kadri, et al.. Epidemiological and genetic characterization of measles virus circulating strains at Marseille, France during 2017–2019 measles outbreak. *Journal of Infection*, 2021, 83 (3), pp.361-370. 10.1016/j.jinf.2021.07.011 . hal-03429566

HAL Id: hal-03429566

<https://amu.hal.science/hal-03429566>

Submitted on 16 Oct 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

1 **Article type: Research article**

2 Article Full-length Title:

3 **Epidemiological and genetic characterisation of measles virus strains**
4 **circulating in Marseille, France during the 2017-2019 measles outbreak**

5 Rita Jaafar^{1,2}, Christine Zandotti^{1,3}, Clio Grimaldier^{1,3}, Maëlia Etoundi³, Ines Kadri^{1,3}, Celine
6 Boschi^{1,2,3}, Priscilla Jardot^{1,3}, Philippe Colson^{1,2,3}, Didier Raoult^{1,2,3}, Bernard La Scola^{1,2,3*},
7 Sarah Aherfi^{1,2,3}

8

9

10

11

12 **Abstract (200 words)**

13 **Objectives.** We attempted to conduct a molecular investigation using next generation sequencing
14 of the measles virus (MeV) strains circulating in Marseille, France, during the last outbreak that
15 occurred between 2017 and 2019.

16 **Methods.** The circulating MeV were isolated from clinical samples using the cell culture method
17 and whole genomes were sequenced by Illumina Miseq Next Generation. Genotyping and
18 comparative analyses were assessed through phylogenetic reconstructions. Clinical and
19 epidemiological data from cases were also recorded.

20 **Results.** A total of 110 MeV strains were isolated in cell culture. Our analysis, based on the
21 whole genome sequences of 98 isolates, confirmed that 93 strains belonged to the genotype D8
22 and 5 to the genotype B3. Phylogenetic analyses revealed four distinct MeV circulating clones in
23 Marseille. Measles mostly occurred in children under the age of five and in adults aged between
24 30 and 50. Measles infection also occurred in two adequately vaccinated cases (two doses). Of
25 63 measles cases on which we had available clinical data, a total of 35 patients were hospitalised
26 and 19 developed complications, including one death.

27 **Conclusions.** Whole Genome Sequencing appears to be a useful tool for more refined genomic
28 characterisation of large measles outbreaks. Vaccination strategies to eradicate measles should be
29 re-evaluated in the current context.

30 **Keywords:** Measles, outbreak, Genotyping, cell culture, Marseille, NGS, whole genome
31 sequencing

32

33

34

35 **TEXT**

36 **Introduction**

37 Measles is caused by a single-stranded RNA virus of the genus *Morbillivirus*, within the family
38 of *Paramyxoviridae*. In 2018, according to the World Health Organization (WHO), more than
39 140,000 people died from measles worldwide[1]. To date, measles is characterised as one of the
40 most contagious diseases in the world, and a single infected individual can cause between 15 and
41 18 secondary cases in a susceptible population[2]. So far, 24 measles virus (MeV) genotypes are

42 currently known to be divided into eight clades from A to H[3]. Those currently circulating in
43 Europe are genotypes B3 and D8[4]. Despite this genomic diversity, all MeV specimens and
44 isolates belong to a single serotype and its infection can be prevented with the MMR (Measles,
45 Mumps, Rubella) vaccine, which also protects against mumps and rubella. Two doses of the
46 MMR vaccine are about 97% effective in preventing measles, and a single dose is about 93%
47 effective[5].

48 In 2001, the WHO launched a programme to eliminate measles. Due to the demonstrated success
49 of measles-associated mortality reduction campaigns and to measles elimination strategies, five
50 of the six WHO regions, including Europe, adopted regional measles eradication goals, initially
51 for 2010[6]. However, unfortunately this deadline could not be maintained [7] and it was
52 extended to 2015 and then to 2020[8]. Although the prevalence of wild-type MeV infection has
53 decreased by more than 90% in Europe[9], measles has not yet been eradicated and has even re-
54 emerged in developed countries, where effective vaccination programmes had already been
55 implemented for many decades[10–12]. In France, the number of measles cases has increased
56 since the beginning of 2018 and during the first semester of 2019, with approximately 1,938
57 cases reported. 562 (29%) cases were hospitalised, 136 (7%) developed pneumonia, two cases
58 developed encephalitis and one patient died[13].

59 Although measles is a vaccine-preventable disease, a timely surveillance of suspected measles
60 cases with a genetic characterisation of the isolates combined with a highly specific and sensitive
61 molecular diagnostic tools is, as described in previous studies, of crucial importance for the
62 global efforts to eliminate MeV and for tracking chains of transmission. With the aim of
63 characterising the MeV genotype, the WHO Global Measles and Rubella Laboratory Network
64 guidelines require the sequencing of the 450 nucleotides of the nucleocapsid (N) gene (N-450) that
65 encode the C terminal region of the protein as the main target for genotyping and the complete coding
66 sequence of the hemagglutinin (H) gene of about 1,845 nucleotides, as a complementary target[14].

67 The MeV genome is a non-segmented negative-strand RNA that encompasses six genes,
68 organised as follows: N, Phosphoprotein (P)/V/C, Matrix (M), Fusion (F), H, and Large
69 polymerase (L). Genotyping only based on the N and H genes may not provide sufficient and
70 valid information for distinguishing endemic from imported MeV[15] and complementary
71 sequencing windows have been proposed as better for monitoring viral evolution and tracking
72 transmission routes [14–17]. Therefore, whole MeV genome sequencing represents an essential

73 and useful tool for a comprehensive exploration of such epidemic resurgences[18]. At the
74 Marseille University hospitals, we aimed to characterise and decipher the molecular
75 epidemiology of circulating MeV strains during the measles outbreaks that occurred in Marseille
76 between 2017 and 2019. Clinical samples which tested positive for MeV by qPCR were first
77 genotyped by Sanger sequencing of the H and N genes, and were then cultured. Isolated virus
78 strains were then sequenced using the NGS Illumina MiSeq platform and the whole genome
79 sequences were analysed.

80 **Materials and Methods**

81 **Study population and clinical samples.** Clinical samples from patients were collected at the
82 IHU Méditerranée Infection during the 2017–2019 measles outbreak in Marseille, France.
83 Clinical samples included saliva, nasopharyngeal, respiratory, urine and blood samples. A
84 serological diagnosis was performed for each patient. IgM and IgG antibodies were detected by
85 Liaison XL automated chemiluminescent immunoassays (Diasorin Inc., Italy). It should be noted
86 that clinical data, including information on vaccination status and complications during the
87 infection were collected.

88 **Molecular tests and Sanger sequencing.** In line with routine practice, MeV RNA was extracted
89 using the EZ1 Virus Mini kit (Qiagen, France) and was detected by the RT-PCR (Reverse-
90 Transcription Polymerase Chain Reaction) assay procedure[19]. PCR-purified products were
91 sequenced. Viral genotypes were conducted based on fragments from the MeV N and/or H
92 genes, obtained by Sanger sequencing, using the Big Dye Terminator cycle (Life Technologies,
93 CA, USA) on the ABI Prism 3130xl genetic analyser (Applied Biosystems, USA). Genotypes
94 were assigned based on sequence identity with the best BLAST hit in the NCBI database.
95 Sequences were aligned using MUSCLE software[20] and phylogenetic reconstructions were
96 performed with the MEGA6 software[21]. Phylogenetic trees based on the nucleocapsid
97 sequences and hemagglutinin sequences, were built using the neighbour-joining method.

98 **Laboratory methods and isolation of the MeV strains.** For the identification of MeV, clinical
99 samples that were positive by PCR were inoculated on cell culture to isolate the virus. The
100 collected samples were first stored at -80°C. Each thawed specimen was then filtrated through
101 0.22 µm microfilters. The cell culture procedure was performed on transfected mammalian
102 African green monkey kidney cells, the Vero/hSLAM cell line (reference: ECACC 04091501),
103 as recommended by the WHO. Vero/hSLAM cells were grown in MEM with Earle's salts

104 enriched with 5% Foetal Bovine Serum (FBS) and 2mM Glutamine while adding 0.4mg/ml of
105 Geneticin (Gibco, Invitrogen, USA). Before virus infection, the cells were seeded at a density of
106 5×10^5 cells/mL. At 80–90% of cell confluency, each well of 12-well plates was inoculated with
107 200 μ L of the filtrated clinical sample and the plate was then incubated at 37°C with 5% CO₂.
108 Cells were then examined for 7–10 days after inoculation using an inverted microscope until a
109 cytopathic effect (CPE) was observed. The cells that did not show CPE were then grown for
110 multiple passages. Strains which were CPE-negative after three passages on cell culture were
111 discarded. For CPE-positive strains, MeV culture was confirmed by PCR testing of 200 μ L of
112 the viral culture medium. The viral suspension was then clarified by centrifugation at 1000g
113 followed by elimination of the pellet containing cell debris and other contaminants. The resulting
114 supernatant containing the virus was divided into several aliquots and stored at -80°C for later
115 use. In addition to clinical samples, the MMR vaccine strain PRIORIX® (GlaxoSmithKline®)
116 was also isolated following the same procedure, and was thus used as a positive control for CPE
117 monitoring of other MeV strains.

118 **Whole genome sequencing.** The viral genomes of the isolated MeV strains were extracted and
119 purified using the RNA easy mini kit (Qiagen, France), and sequenced using the Illumina MiSeq
120 platform and the MiSeq Reagent Kit v2 (500-cycles), according to the manufacturer's protocols.
121 Paired-end libraries for the MiSeq platform were prepared based on Nextera XT DNA Library
122 Preparation Kit (96 samples), according to the manufacturer's protocols. Low-quality reads were
123 removed and reads were mapped on 152 representative MeV genomes using CLC Genomics
124 Workbench software v7.5. Consensus sequences were provided after the read-mapping process
125 by removing the regions not covered and joining the sequences obtained after removal. In
126 addition, we applied vote resolution to handle conflicts. Genotypes were determined by the best
127 mapped reference genome, taking into account both average coverage and consensus sequence
128 length.

129 **Genotyping analysis.** For the genotyping step of MeV isolates, the dataset in this study
130 consisted of the translated sequences of MeV genes. Sequences of each gene were translated into
131 amino acids using EMBOSS Transeq. The included strains represent the Marseille MeV strains
132 isolated in comparison with reference strains defined by the WHO[22]. A phylogenetic
133 reconstruction was conducted on the nucleoprotein peptide sequences of these strains, as

134 recommended by the WHO for genotype confirmation[25]. The same phylogeny was established
135 for of the N, M, F and H amino-acid sequences, concatenated together.

136 **Genomic and phylogenetic analysis.** Whole genome sequence comparative analysis was
137 performed independently for each genotype and for each gene, and included all the sequences
138 belonging to the specific genotype studied available on the NCBI database. Sequences were
139 aligned using the MUSCLE software[20] and phylogenetic trees were built with the maximum
140 likelihood method using the FastTree v2.1.10 software[23]. The robustness of the groupings was
141 assessed by bootstrap resampling of 1,000 replicates and only significant bootstrap values
142 (>70%) were displayed. Phylogenetic tree styling and advanced tree elements editing were
143 applied using iTOL v6.1.1[24].

144 **Results**

145 **Laboratory confirmation of measles cases.** Between 2017 and 2019, a total of 1,494 samples
146 corresponding to 1,126 individuals in Marseille with suspected measles infection were tested by
147 qPCR. As a result, 330 clinical samples related to 212 patients tested positive for MeV by PCR
148 (Supplementary file 1a).

149 **Age group distribution of positive measles cases.** MeV infection was observed in patients aged
150 from 0 to 62 years with a mean age of 17 years. The highest measles cases incidence occurred in
151 children under the age of five, representing 69 cases (32.9%), and in patients aged between 30
152 and 50, representing 52 patients (25%) (**Fig.1a**). The youngest patient was a newborn infected *in*
153 *utero*. The mother tested positive for measles two days before delivery and was diagnosed with
154 positive anti-MeV IgM while the IgG had shown to be at the limit of positivity seven days after
155 PCR diagnosis. The vaccine status of the mother was unknown. The new-born tested positive
156 for measles after a PCR test was performed on its umbilical cord blood three days after birth. At
157 this time, he presented no evidence of IgG. Two days after, this child was also tested positive for
158 measles by PCR on a pharyngeal swab. The child was given intravenous immunoglobulin (IVIG)
159 at 200 mg/kg, did not show any sign of congenital measles, and was discharged five days after
160 the first positive PCR diagnosis. Clinical examination was still normal at 10 days of life
161 (**Supplementary file 2**).

162 **Clinical investigation of the study population.** Clinical data were available for 70 patients. Of
163 these, 34 were hospitalised and 19 presented complications (**Supplementary file 2**). These
164 complications included pneumopathy (21 cases) and hepatitis (6 cases). In addition, acute

165 median otitis and conjunctivitis occurred in seven cases, of which five were children. Besides, a
166 30-year-old man reported cognitive impairment including attention and concentration disorders
167 three weeks after the rash, but biological examination of his cerebrospinal fluid and cerebral
168 tomography three months later showed no anomalies. One fatal case of measles occurred in a 26
169 year-old kidney transplant recipient man who developed severe acute respiratory distress
170 syndrome with ground-glass opacities visualised by CT-TDM, and complicated with a septic
171 shock. He suffered from Job syndrome, consisting of hyper-IgE production and an IgG deficit.
172 The patient was receiving immune-suppressive therapy which consisted of mycophenolate
173 mofetil (250 mg twice per day), prednisolone (7.5 mg per day) and tacrolimus (10 mg per day).
174 The MeV infection was diagnosed at a late stage by PCR on his broncho-alveolar liquid, two
175 weeks after the onset of symptoms, and by a serological test that showed positive IgG and IgM
176 for measles at the same time. Bacterial culture and other infectious agents tested by PCR were
177 negative on the same sample. Urinary *Legionella* and *Streptococcus pneumoniae* antigens were
178 negative. Multiple probabilist anti-infective therapies were tentatively given, including
179 vancomycin, voriconazole, cotrimoxazole, spiramycin, meropenem, and linezolid. One day after
180 the diagnosis, the patient received ribavirin and vitamin A. One week later, intravenous
181 immunoglobulins were administered, but the patient died 18 days after being diagnosed with
182 MeV (**Supplementary file 2**).

183 **Patients' vaccine status data.** About 74% (113 of 152 patients) of patients in the study
184 population had an unknown vaccine status, either because clinical samples were sent from other
185 hospitals, or due to the lack of information provided by the patient. A total of 30 cases (20 %)
186 were unvaccinated: six were infants under the age of one (before the recommended age of the
187 first vaccine dose), and two which were due to vaccine refusal by parents
188 (**Fig.1b**)(**Supplementary file 2**). A total of eight people (5%) were vaccinated (**Fig. 1b**),
189 including six (4%) who had received only one dose and two (1%) who had received two doses of
190 the MMR vaccine (**Supplementary file 2**). Three of the vaccinated cases (cases 14, 19, and 21)
191 had a serum collected concomitantly to the measles diagnosis, which was tested for anti-MeV
192 IgM and IgG. Two out of the 19 cases that received two doses and 21 that had received one dose
193 of MMR vaccine harboured anti-MeV IgG antibodies (**Supplementary file 3**).

194 **Sanger sequencing of MeV samples and routine genotyping.** N and H gene sequences were
195 tentatively performed for 91 cases. A sequence of the N and the H genes was obtained for 37 and

196 45 patients, respectively, and a sequence from at least one of these two genes was obtained for
197 73 (80.2%) of the 91 cases (**Supplementary file 9**). Based on phylogenetic analyses, the MeV
198 genotype was A, B3 and D8 in two (2.7%), 17 (23.3%) and 54 (73.9%) cases, respectively
199 (**Supplementary file 4**). Phylogenetic reconstructions showed that sequences from the D8
200 genotype closely clustered together, apart from the best blast hits sequences from the D8
201 genotype. The same topology was noted for sequences identified as belonging to the B3
202 genotype that formed a single cluster slightly apart from other B3 sequences. Genotype A was
203 identified in two post-vaccinal pediatric infections in two 24- and 13-month-old children, case 55
204 and 57 respectively (**Supplementary file 2**). The first (case 55) had received one dose of the
205 vaccine, three weeks before the appearance of a slightly febrile rash. The second (case 57)
206 received a first dose of the vaccine seven days before the appearance of bilateral conjunctivitis.
207 Two days later, he developed a rash. In this second case, serum was available and serological
208 testing showed anti-measles IgG negativity and IgM positivity, confirming the absence of
209 maternal immunity against measles.

210 **Isolation of MeV strains.** A total of 257 clinical samples which were positive by PCR for MeV
211 were inoculated in cell cultures resulting in 110 positive samples grown in cell culture.
212 (**Supplementary file 5**) As these samples were related to 99 patients, we ultimately identified a
213 total of 99 MeV isolates. To note, respiratory samples had the highest yield (58% of inoculated
214 samples) of positivity in cell culture (**Supplementary file 1b**).

215 **Genomic characterisation of the MeV isolates.** After discarding contaminated sequencing data,
216 98 complete MeV genome sequences were successfully generated from the MeV isolates
217 (**Supplementary file 9**). Phylogenetic analyses of the carboxyl-terminal 150 amino-acid encoded
218 by the N-450 hypervariable region of the nucleoprotein gene, indicated that these isolates belong
219 to the genotypes D8 and B3 (**Fig.2**). The genotype D8 was predominant with 93 isolates
220 clustering with both MVi/Haridwar.IND/06.14/2[D8] (GenBank Accession No: MN125030.1)
221 and MVs/London.GBR/22.12/3[D8] (GenBank Accession No: KT732231.1) reference
222 sequences, whereas only five isolates belonged to the genotype B3, the phylogenetically closest
223 reference sequences being MVs/Padova.ITA/24.17/1[B3] (GenBank Accession No:
224 MK513622.1) and MVi/Manchester.GBR/31.13[B3] (GenBank Accession No: KT732215)
225 (**Fig.2**). The evolutionary relationships based on whole genome sequences between all the
226 Marseille isolates revealed four clusters: three for the genotype D8 (clusters named D8-A, D8-B

227 and D8-C) and one for the genotype B3 (**Fig.3**). Temporal clustering showed that the D8-C
228 cluster was the first to appear in May 2017 (**Fig.4, Supplementary file 9**).The D8-C cluster
229 tended to be the most widespread over the entire 2017–2019 outbreak in Marseille, with waves
230 of interrupted reappearance. The D8-B cluster was likely to have been circulating for only four
231 months at the end of 2018. However, MeV strains from the D8-A cluster were predominant
232 during 2019, and overlapped from June until October 2019, with those from the D8-C cluster,
233 and also with those of the B3 genotype, from May until the first half of August 2019.

234 **Potential transmission pathways.** Further phylogenetic analyses on whole genome sequences
235 including other European and worldwide MeV reference strains from genotypes D8 and B3,
236 enabled us to refine the analysis further (**Fig.5, Fig.6**).The sequences of the D8-B cluster (six
237 strains) were closely related to the MVs/California.USA/38.19 (GenBank Accession No:
238 MT789845.1) and also clustered with Italian strains identified in Ancona, Padova and Venezia
239 (GenBank Accession No: MH173047.1, MK513623.1, and MK513627.1, respectively) in 2017.
240 All 59 of the complete virus sequences of the D8-A cluster were phylogenetically linked with a
241 group of 27 Californian D8 strains that were circulating in 2019 and to the
242 MVs/Zhoushan.CHN/09.19[D8] (GenBank Accession No: MT738550.1) that circulated in China
243 during the same year. For the D8-C cluster, which included 41 Marseille MeV strains, seven
244 strains were identified in the 2019 period and were phylogenetically linked to the
245 MVs/California.USA/29.19 sequence (Genbank Accession No: MT789841.1). It should be noted
246 that all the sequences of this cluster were also related to three strains from Korea
247 (MVi/Geoje.KOR/41.16, GenBank Accession No: MF496201.1), Australia
248 (MVs/Brisbane.AUS/33.15, GenBank Accession No: MH638233.1) and Brazil
249 (MVs/Roraima.BRA/31.18, GenBank Accession No:MK161348.1). Finally, the five Marseille
250 MeV strains from the B3 genotype composed a unique cluster that is closely related to the
251 Californian sequence MVs/California.USA/50.19 (GenBank Accession No: MT789809.1)
252 (**Fig.6**). In addition, we noticed that it also clustered with multiple strains from Europe such as
253 Manchester-UK and Padova-Italy but, most importantly, the Marseille MeV strains from the B3
254 genotype appear to have a potential close-evolutionary relationship with two French MeV strains
255 that were identified in Calais (GenBank Accession No: KX838946.2) and Tours (Genbank
256 Accession No: MN893225.1), in 2016 and 2018, respectively (represented with a grey
257 background in Fig.6). Phylogenetic reconstruction based only on the N-450 (450 bp) region also

258 showed that the B3 isolates were congruently related to the Calais and Tours sequences, but also
259 to other French strains from Saint Denis (2017; GenBank Accession No: MH129056 and
260 MH161350) and from Mamoudzou, in the French overseas territories of Reunion and Mayotte
261 (2018; GenBank Accession No: MK733316 and MK733315) (**Supplementary file 6**). In
262 addition, the Marseille MeV strains in the D8-C cluster were related to a French strain identified
263 in Bordeaux in 2018 (GenBank: MH796650; MH796651).

264 **Discussion**

265 This study was conducted in order to establish the genetic and epidemiological description of the
266 measles outbreaks that occurred in Marseille, France. Between 2017 and 2019, 1,126 patients
267 tested positive for MeV at the Marseille University Hospitals, in southeastern France
268 (**Supplementary material 1a**). This study, which is the first to our knowledge combining cell
269 culture with whole genome analysis by Next Generation Sequencing, made it possible to identify
270 four circulating clones of MeV in Marseille.

271 In 1998, the WHO issued guidelines for a unified nomenclature for identifying wild-type MeV
272 and for its genotypic description. Recently, the molecular characterisation of MeV has been an
273 essential part of measles surveillance. The Next Generation Sequencing performed here, made it
274 possible to identify genotype-specific spatio-temporal clusters (**Fig.4**) responsible for the
275 currently circulating MeV clones, and provided a more refined analysis

276 Only eight genotypes have been in circulation since 2009[3], the most prevalent of which in
277 France were D5 in 2008 and D4 in 2011, suggesting a rapidly evolving dynamic of circulating
278 measles strains[25]. Our work demonstrated that the D8 genotype was the most prevalent,
279 followed by the B3 genotype. These data are in line with the literature, since B3 and D8
280 genotypes are those that have almost exclusively been in circulation since 2015[4]. Overall, we
281 observed the presence of three different D8 clusters and only one B3 cluster (**Fig.3**).The NGS
282 enabled us to link these clusters (**Fig.5 and Fig.6**) to clones that have recently circulated around
283 the world (Europe, North America, South America, Oceania and Asia). In addition, these strains
284 can be defined as unique clones and they seem to be chronologically-linked to the potential
285 chains of transmission of MeV that circulated between 2017 and 2019 worldwide. However, as
286 few laboratories perform whole genome MeV sequencing, interpretation of the results of this
287 study is limited by the scarcity of data available on whole genome sequences around the world.
288 Additionally, these clusters contain strains from same family members as shown in

289 **supplementary file 7**, which explain the rapid transmission and the high incidence in specific
290 periods (**Fig.4**).

291 Despite the fact that several public health measures have been implemented in France since 2005
292 to limit the spread of MeV, including the introduction of mandatory reporting and the target of
293 95% vaccine coverage at two years of age, France has experienced several measles outbreaks,
294 first in the 2008-2011[25] period then from the end of 2017 until the end of 2019[4]. The latest
295 epidemic in France began in the New-Aquitaine region with 50% of all reported cases that
296 occurred between October 2017 and September 2018, rapidly spreading to almost whole of
297 France[13]. In March 2018, a study in Portugal reported on a chain of transmission that
298 originated with a French citizen coming from this same region and who was also the source of
299 infection of three additional vaccinated cases in his environment[26,27]. Our findings
300 (**Supplementary file 6**) therefore also reveal a strong likelihood between the D8-C cluster of
301 MeV circulating in Marseille in 2018 and in Bordeaux (south-west France). The first clone
302 identified in Marseille during this period (June 2017) was the D8-C cluster, which continued
303 circulating until September 2019, and closely-related sequences were detected in France and
304 Portugal (**Supplementary file 6**).

305 It is commonly known that MeV is serologically monotypic. However, data on the efficacy of
306 the vaccine (containing an inactivated strain of genotype A MeV) on the other genotypes is
307 lacking. During the outbreak, we observed 20% of confirmed measles cases among patients who
308 were previously immunised, from the patients with a known vaccine history. Primary and
309 secondary failure were already described in previous studies. Secondary failure, characterised by
310 the development of specific IgG antibodies after vaccination but with a total lack of protection
311 against any subsequent infection with the wild-type virus, are reported in about 5% at 10–15
312 years after the patients are immunised [3]. However, anti-MeV IgG (**Supplementary file 3**)
313 detected at the first beginning of the infection for two vaccinated patients, shows that vaccine-
314 induced antibodies are, at least in some cases, not protective against the measles infection. The
315 lack of a humoral response after vaccination was not seen in this study but has previously been
316 described[28]. It is noteworthy that naturally acquired immunity may induce higher titres of
317 protective antibodies than vaccine-induced immunity[29].

318 Despite a high vaccination coverage in France, analyses of the demographics of the measles
319 outbreak revealed immunity gaps in children under the age of five and in adults over the age of

320 30, representing 32.9% and 25% of measles cases, respectively (**Fig.1b**). While infants from
321 vaccinated mothers lose the protective maternal antibodies after only 0.97 months and those from
322 naturally immune mothers lose them after 3.78 months [30], they remain ineligible for
323 vaccination, according to the current schedules. The first dose of the MeV vaccine is
324 recommended at the age of nine months, or at 12–15 months in countries in which the risk of
325 measles is low. These data suggest that the occurrence of measles outbreaks in France may not
326 rely primarily on insufficient vaccine coverage, but may be also related to the inappropriate
327 targeting of vaccine immunisation [4]. In addition, in May 2019, the Californian department of
328 public health recommended, for infants aged between six and 11 months who will be travelling
329 internationally, or who belong to a community affected by a sustained, community-wide MeV
330 transmission, one extra early dose of MMR vaccine [31,32]. This recommendation was
331 subsequently approved by the Centers for Diseases Control and Prevention [33]. The region of
332 Tianjin in China also uses a three-dose MMR regimen, the first being recommended at eight
333 months, the second at 18–24 months, and again at 4–6 years [34]. In that region, the proportion
334 of measles infections in infants under the age of one represents 8% of the total. Such a vaccine
335 strategy, with a first dose at six months of age, followed by the two currently recommended
336 doses, may therefore be relevant, especially in the context of outbreaks.

337 Sanger sequencing based on only the N and/or H genes sequences could not precisely identify
338 the different clusters provided by phylogenetic analysis from the sequences provided by Next
339 Generation Sequencing. In France, only two whole genome sequences were found in the NCBI
340 Genbank nt database referring to the MeV strains in Calais[35] and Tours[36], as obtained by the
341 conventional Sanger sequencing method. This study may make a significant contribution to the
342 introduction of MeV whole genome sequencing to the international protocol for a more in-depth
343 measles genomic characterisation and better tracking of measles transmission.

344 Our work, based on virus isolates, provides for the re-introduction of isolation-on-cell-culture as
345 a useful tool for the characterisation and comprehensive study of the molecular epidemiology of
346 circulating strains of MeV, especially in the case of large epidemics. However, this kind of study
347 may not be applicable on a routine basis in all laboratories. Indeed, the high cost of Next
348 Generation Sequencing, combined with the high level of technicity required for the time-
349 consuming cell-culture methods, make this proposed procedure less feasible than standard
350 genotyping for the majority of surveillance and diagnostic laboratories.

351 Finally, the occurrence of different strains circulating at the same time raises the risk of
352 subsequent transmission and points to the need for constant molecular surveillance for the early
353 identification and monitoring of the spread of MeV. The development of MeV whole genome
354 sequencing by NGS on the currently circulating measles strains appears to have potential for the
355 future and needs to be implemented more widely in routine diagnosis laboratories. This approach
356 appears crucial to enhance our knowledge of MeV molecular epidemiology and the resurgence
357 of measles, with the aim of taking the most appropriate preventive measures to limit measles
358 outbreaks.

359

360 **ACKNOWLEDGMENTS**

361 We would like to thank Emilie Javelle for her review of the manuscript, and for her advises.

362

363 **FUNDINGS**

364 This work was supported by the French Government under the “Investments for the Future” program
365 managed by the National Agency for Research (ANR), Méditerranée-Infection 10-IAHU-03 and was also
366 supported by Région Provence-Alpes-Côte d’Azur and European funding Fonds Européen de
367 Développement Régional - Plateformes de Recherche et d’Innovation Mutualisées Méditerranée Infection
368 (FEDER PRIMMI).

369

370

371 **Figures**

372 **Fig 1.** Clinical data of Measles confirmed cases: **(a)** Distribution of all measles cases ($N_a=212$)
373 by age group during the 2017-2019 outbreak in Marseille, France. Each color corresponds to an
374 age group as shown in the legend on the right. **(b)** Percentage of measles cases by vaccination
375 status ($N_b=155$). The study of vaccine uptake history was provided only for patients included in
376 the study population as mentioned previously (Materials and method section).

377 **Fig 2.** Genotyping of MeV strains circulating in Marseille, France, between 2017 and 2019.
378 Based on the full-length 150 amino acid sequence of the N-450 (450 bp) Nucleoprotein region. A
379 maximum likelihood phylogenetic reconstruction by FastTree v2.1.10, was performed with 98
380 Marseille-MeV strains and the 24 genotype-references from the WHO's list [22] for genotyping.
381 Only the Marseille-MeV strains are highlighted. Highlight colours are indicated in the legend on
382 the right. The MMR vaccine-isolated virus strain is indicated with a grey font.

383 **Fig 3.** Phylogenetic reconstruction using the maximum-likelihood evolutionary relationship
384 method, using FastTree v2.1.10, of the 98 Marseille-MeV strains, based on whole genome
385 nucleotide sequences. The final alignment included 15,358 nucleotide positions. The four main
386 circulating strains clusters are shown in different colours.

387 **Fig 4.** Graph showing the monthly evolution of each MeV transmission cluster as identified
388 between January 2017 and February 2020 in Marseille, France in our laboratory (the number of
389 strains is shown on the y-axis and time by month is shown on the x-axis). Clusters D8: A, B and
390 C are shown, respectively, in green, orange and red. The single B3 cluster is shown in dark blue.
391 A dataset table of strain numbers by month and cluster is also shown below the graph.

392 **Fig 5.** Circular phylogenetic tree built with FastTree v2.1.10, based on the whole genome
393 sequences of the 93 Marseille-MeV strains from the D8 genotype and 20 MeV complete genome
394 sequences from the D8 genotype available in the NCBI database. The final alignment is
395 composed of 15,424 nucleotide positions. NCBI sequences are coloured based on the continent
396 in which the strain was identified. The Marseille-MeV strains are highlighted in yellow.

397 **Fig 6.** Circular representation of the phylogenetic tree, built with FastTree v2.1.10, based on
398 whole genome sequences of the five Marseille-MeV strains from the B3 genotype with the 76
399 MeV complete genome sequences from the B3 genotype available in the NCBI database. The

400 final alignment was composed of 15,427 nucleotide positions. NCBI sequences are coloured
401 based on the continent in which the strain was identified. The Marseille-MeV strains are
402 highlighted in yellow.

403

404

405

406 **REFERENCES**

- 407 1. More than 140,000 die from measles as cases surge worldwide. Available at:
408 [https://www.who.int/news/item/05-12-2019-more-than-140-000-die-from-measles-as-cases-surge-](https://www.who.int/news/item/05-12-2019-more-than-140-000-die-from-measles-as-cases-surge-worldwide)
409 [worldwide](https://www.who.int/news/item/05-12-2019-more-than-140-000-die-from-measles-as-cases-surge-worldwide). Accessed 26 October 2020.
- 410 2. Bellini WJ, Icenogle JP. Measles and Rubella Viruses. In: Manual of Clinical Microbiology. John
411 Wiley & Sons, Ltd, 2015: 1519–1535. Available at:
412 <https://onlinelibrary.wiley.com/doi/abs/10.1128/9781555817381.ch87>. Accessed 26 October 2020.
- 413 3. Moss WJ. Measles. *The Lancet* **2017**; 390:2490–2502.
- 414 4. Javelle E, Colson P, Parola P, Raoult D. Measles, the need for a paradigm shift. *Eur J Epidemiol*
415 **2019**; 34:897–915.
- 416 5. About Measles Vaccination | Vaccination and Immunizations | CDC. 2020. Available at:
417 <https://www.cdc.gov/vaccines/vpd/measles/index.html>. Accessed 26 October 2020.
- 418 6. WHO | Sixty-third World Health Assembly closes after passing multiple resolutions. World
419 Health Organization, Available at:
420 https://www.who.int/mediacentre/news/releases/2010/wha_closes_20100521/en/. Accessed 26 October
421 2020.
- 422 7. Santibanez S, Hübschen JM, Muller CP, et al. Long-term transmission of measles virus in Central
423 and continental Western Europe. *Virus Genes* **2015**; 50:2–11.
- 424 8. Orenstein WA, Hinman A, Nkowane B, Olive JM, Reingold A. Measles and Rubella Global
425 Strategic Plan 2012–2020 midterm review. *Vaccine* **2018**; 36:A1–A34.
- 426 9. Holzmann H, Hengel H, Tenbusch M, Doerr HW. Eradication of measles: remaining challenges.
427 *Med Microbiol Immunol (Berl)* **2016**; 205:201–208.
- 428 10. Baccolini V, Sindoni A, Adamo G, et al. Measles among healthcare workers in Italy: is it time to
429 act? *Hum Vaccines Immunother* **2020**; 0:1–10.
- 430 11. Eichner L, Wjst S, Brockmann SO, Wolfers K, Eichner M. Local measles vaccination gaps in
431 Germany and the role of vaccination providers. *BMC Public Health* **2017**; 17. Available at:
432 <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5557556/>. Accessed 2 December 2020.
- 433 12. Dunn JJ, Baldanti F, Puchhammer E, Panning M, Perez O, Harvala H. Measles is Back –
434 Considerations for laboratory diagnosis. *J Clin Virol* **2020**; 128:104430.
- 435 13. SPF. Bulletin épidémiologique rougeole. Données de surveillance 2019. Available at: /maladies-
436 et-traumatismes/maladies-a-prevention-vaccinale/rougeole/documents/bulletin-national/bulletin-
437 epidemiologique-rougeole.-donnees-de-surveillance-2019. Accessed 3 December 2020.
- 438 14. The role of extended and whole genome sequencing for tracking transmission of measles and
439 rubella viruses: report from the Global Measles and Rubella Laboratory Network meeting, 2017. *Releve*
440 *Epidemiol Hebd* **2018**; 93:55–59.
- 441 15. Penedos AR, Myers R, Hadeif B, Aladin F, Brown KE. Assessment of the Utility of Whole
442 Genome Sequencing of Measles Virus in the Characterisation of Outbreaks. *PLOS ONE* **2015**;
443 10:e0143081.

- 444 16. Kessler JR, Kremer JR, Shulga SV, et al. Revealing New Measles Virus Transmission Routes by
445 Use of Sequence Analysis of Phosphoprotein and Hemagglutinin Genes. *J Clin Microbiol* **2011**; 49:677–
446 683.
- 447 17. Bianchi S, Canuti M, Ciceri G, et al. Molecular Epidemiology of B3 and D8 Measles Viruses
448 through Hemagglutinin Phylogenetic History. *Int J Mol Sci* **2020**; 21:4435.
- 449 18. Beerenwinkel N, Günthard HF, Roth V, Metzner KJ. Challenges and opportunities in estimating
450 viral genetic diversity from next-generation sequencing data. *Front Microbiol* **2012**; 3. Available at:
451 <https://www.frontiersin.org/articles/10.3389/fmicb.2012.00329/full>. Accessed 26 October 2020.
- 452 19. Godefroy R, Chaud P, Ninove L, et al. Measles outbreak in a French Roma community in the
453 Provence-Alpes-Côte d'Azur region, France, May to July 2017. *Int J Infect Dis* **2018**; 76:97–101.
- 454 20. Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput.
455 *Nucleic Acids Res* **2004**; 32:1792–1797.
- 456 21. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary
457 Genetics Analysis Version 6.0. *Mol Biol Evol* **2013**; 30:2725–2729.
- 458 22. WHO | Manual for the Laboratory-based Surveillance of Measles, Rubella, and Congenital
459 Rubella Syndrome. World Health Organization, Available at:
460 http://www.who.int/immunization/monitoring_surveillance/burden/laboratory/manual/en/. Accessed 26
461 October 2020.
- 462 23. Price MN, Dehal PS, Arkin AP. FastTree 2 – Approximately Maximum-Likelihood Trees for
463 Large Alignments. *PLOS ONE* **2010**; 5:e9490.
- 464 24. Letunic I, Bork P. Interactive Tree Of Life (iTOL) v4: recent updates and new developments.
465 *Nucleic Acids Res* **2019**; 47:W256–W259.
- 466 25. Antona D, Lévy-Bruhl D, Baudon C, et al. Measles Elimination Efforts and 2008–2011 Outbreak,
467 France. *Emerg Infect Dis* **2013**; 19:8.
- 468 26. Bernadou A, Astrugue C, Méchain M, et al. Measles outbreak linked to insufficient vaccination
469 coverage in Nouvelle-Aquitaine Region, France, October 2017 to July 2018. *Eurosurveillance* **2018**; 23.
470 Available at: <https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2018.23.30.1800373>.
471 Accessed 7 May 2021.
- 472 27. Augusto GF, Cruz D, Silva A, et al. Challenging measles case definition: three measles outbreaks
473 in three Health Regions of Portugal, February to April 2018. *Eurosurveillance* **2018**; 23:1800328.
- 474 28. Paunio M, Hedman K, Davidkin I, et al. Secondary measles vaccine failures identified by
475 measurement of IgG avidity: high occurrence among teenagers vaccinated at a young age. *Epidemiol
476 Infect* **2000**; 124:263–271.
- 477 29. Christenson B, Böttiger M. Measles antibody: comparison of long-term vaccination titres, early
478 vaccination titres and naturally acquired immunity to and booster effects on the measles virus. *Vaccine*
479 **1994**; 12:129–133.
- 480 30. Leuridan E, Hens N, Hutse V, Ieven M, Aerts M, Van Damme P. Early waning of maternal
481 measles antibodies in era of measles elimination: longitudinal study. *BMJ* **2010**; 340:c1626.

- 482 31. Patel M, Lee AD, Redd SB, et al. Increase in Measles Cases — United States, January 1–April
483 26, 2019. *MMWR Morb Mortal Wkly Rep* **2019**; 68:402–404.
- 484 32. Measles Information for Clinicians - Public Health Department - For Providers - County of Santa
485 Clara. Available at: <https://www.sccgov.org/sites/phd-p/Diseases/Measles/Pages/measles-home.aspx>.
486 Accessed 17 December 2020.
- 487 33. Routine MMR Vaccination Recommendations | For Providers | CDC. 2019. Available at:
488 <https://www.cdc.gov/vaccines/vpd/mmr/hcp/recommendations.html>. Accessed 17 December 2020.
- 489 34. Montgomery JP, Zhang Y, Carlson B, Ewing S, Wang X, Boulton ML. Measles Vaccine
490 Coverage and Series Completion among Children 0 to 8 Years of Age in Tianjin, China. *Pediatr Infect*
491 *Dis J* **2015**; 34:289–295.
- 492 35. Dina J, Hamel J, Antona D, Vabret A. Complete Genome Sequence of a Wild-Type Measles
493 Virus Isolated during a 2016 Winter Outbreak in a Refugee Settlement in Calais, France. *Genome*
494 *Announc* **2017**; 5.
- 495 36. Rodriguez C, Gouilh MA, Weiss N, et al. Fatal Measles Inclusion-Body Encephalitis in Adult
496 with Untreated AIDS, France - Volume 26, Number 9—September 2020 - *Emerging Infectious Diseases*
497 *journal* - CDC. Available at: https://wwwnc.cdc.gov/eid/article/26/9/20-0366_article. Accessed 2
498 December 2020.
- 499

Fig.1

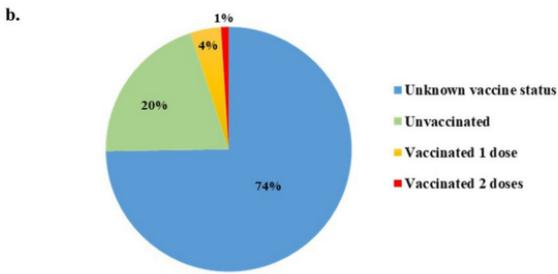
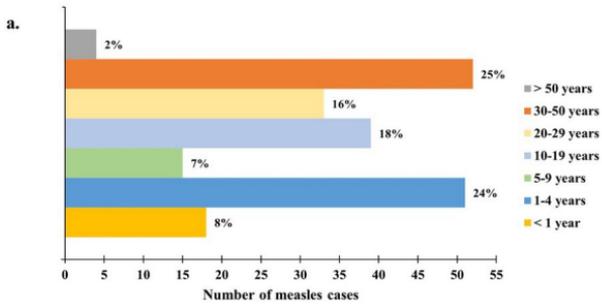


Fig.3

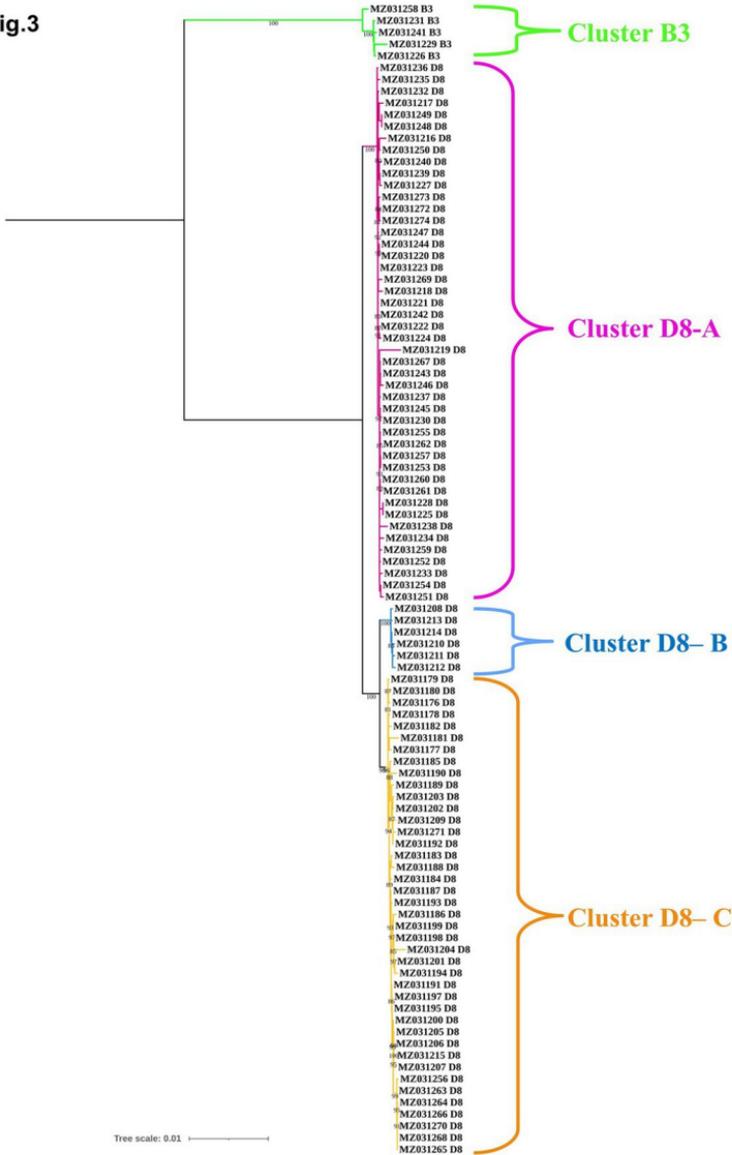


Fig.5

