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

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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

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Version of Record: https://www.sciencedirect.com/science/article/pii/S0163445321003522
Manuscript_04bc14d76989f9f075d9f929b69888bf

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**

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Abstract (200 words)

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

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

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

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

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

TEXT

Introduction

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

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

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

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

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

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

Materials and Methods

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

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

Laboratory methods and isolation of the MeV strains. For the identification of MeV, clinical samples that were positive by PCR were inoculated on cell culture to isolate the virus. The collected samples were first stored at -80ºC. Each thawed specimen was then filtrated through 0.22 µm microfilters. The cell culture procedure was performed on transfected mammalian African green monkey kidney cells, the Vero/hSLAM cell line (reference: ECACC 04091501), 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 x 10^5 cells/mL. At 80–90% of cell confluency, each well of 12-well plates was inoculated with
107 200 µL of the filtrated clinical sample and the plate was then incubated at 37°C with 5% CO2.
108 Cells were then examinated 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 µL 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

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

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

Results

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

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

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

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

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

Sanger sequencing of MeV samples and routine genotyping. N and H gene sequences were 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

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

Discussion

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

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

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

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

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

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

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

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

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

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

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

ACKNOWLEDGMENTS

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

FUNDINGS

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

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
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405

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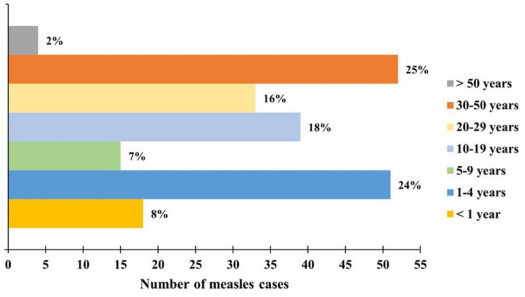
Fig.1

a.


2%
25%
16%
18%
7%
24%
8%
> 50 years
30-50 years
20-29 years
10-19 years
5-9 years
1-4 years
< 1 year
0
5
10
15
20
25
30
35
40
45
50
55
Number of measles cases

b.

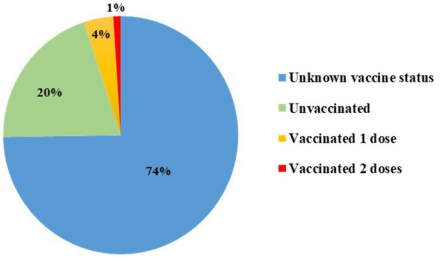
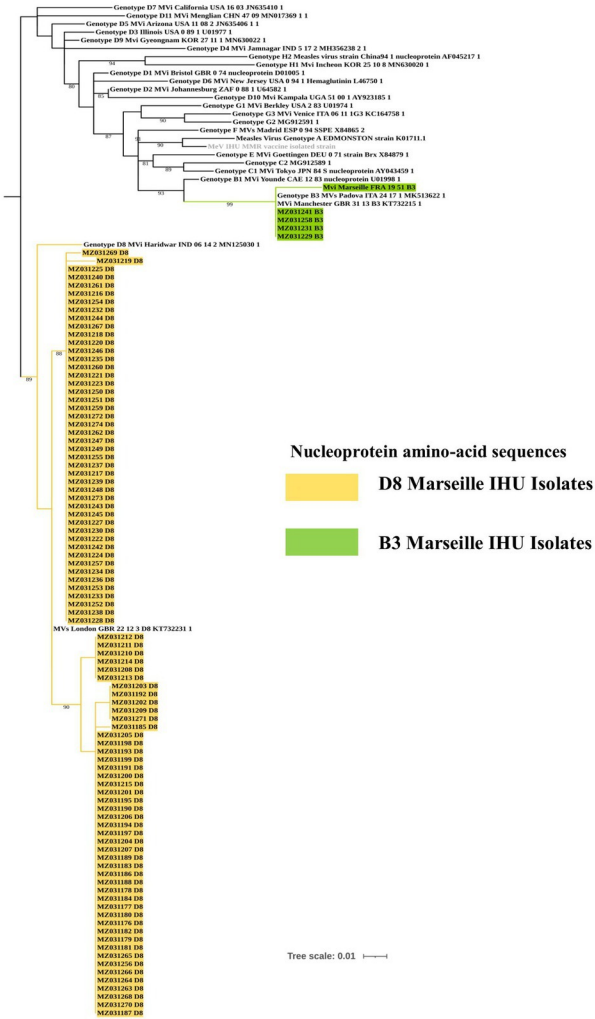

1%
4%
20%
74%
Unknown vaccine status
Unvaccinated
Vaccinated 1 dose
Vaccinated 2 doses

Fig.2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Genotype D11 MVi Menglian CHN 47 09 MN017369 1 1
Genotype D5 MVi Arizona USA 11 08 2 JN635406 1 1
Genotype D3 Illinois USA 8 89 1 U01977 1
Genotype D9 Mvi Gyeongnam KOR 27 11 1 MN630022 1
Genotype D4 MVi Jamnagar IND 5 17 2 MH356238 2 1
Genotype H2 Measles virus strain China94 1 nucleoprotein AF045217 1
Genotype H1 Mvi Incheon KOR 25 10 8 MN630020 1
Genotype D1 MVi Bristol GBR 0 74 nucleoprotein D01005 1
Genotype D6 MVi New Jersey USA 0 94 1 Hemaglutinin L46750 1
Genotype D2 MVi Johannesburg ZAF 0 88 1 L46582 1
Genotype D10 Mvi Kampala UGA 51 00 1 AY923185 1
Genotype G1 MVi Berkley USA 2 83 U01974 1
Genotype G3 MVi Venice ITA 06 11 1G3 KC164758 1
Genotype G2 MG912591 1
Genotype F MVs Madrid ESP 0 94 SSPE X84865 2
Measles Virus Genotype A EDMONSTON strain K01711.1
SSPE (H1) NDMB vaccine isolate(Urasoe)
Genotype E MVi Goettingen DEU 0 71 strain Brx X84879 1
Genotype C2 MG912589 1
Genotype C1 MVi Tokyo JPN 84 5 nucleoprotein AY043459 1
Genotype B1 MVi Yoonde CAE 12 83 nucleoprotein U01998 1
Mvi Marseille IHUA 19 51 B3
Genotype B3 MVs Padova ITA 24 17 1 MK513622 1
MVi Manchester GBR 31 13 B3 KT732215 1
MZ031241 B3
MZ031258 B3
MZ031231 B3
MZ031229 B3
Genotype D8 MVi Haridwar IND 06 14 2 MN125030 1
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MZ031219 D8
MZ031225 D8
MZ031240 D8
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MZ031270 D8
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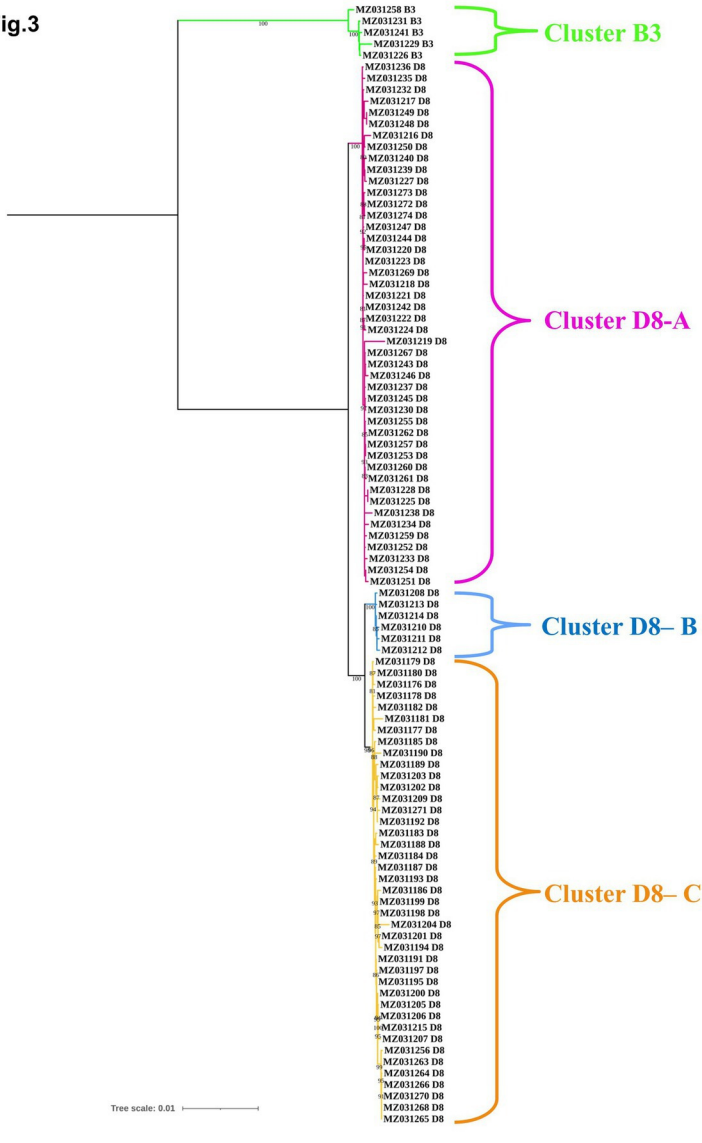
Nucleoprotein amino-acid sequences
D8 Marseille IHU Isolates
B3 Marseille IHU Isolates
Tree scale: 0.01

Fig.3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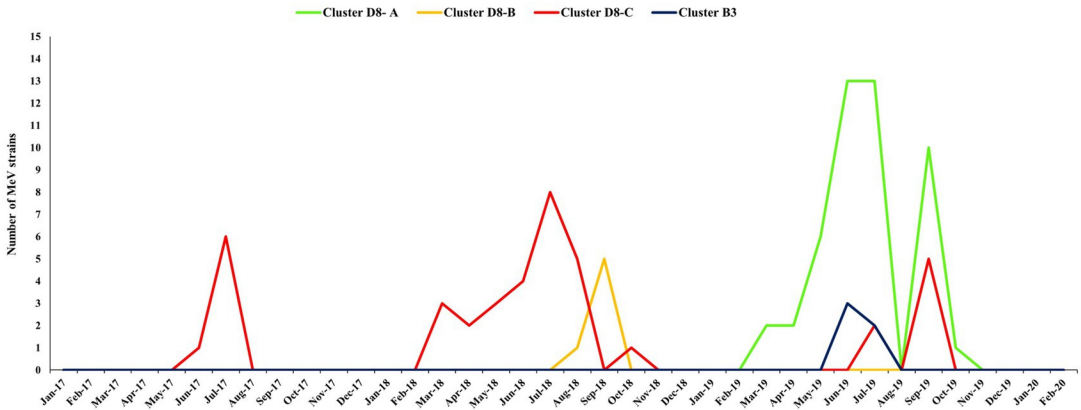
Cluster B3
Cluster D8-A
Cluster D8– B
Cluster D8– C
Tree scale: 0.01

Fig.4



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Cluster D8- A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	6	13	13	0	10	1	0	0	0	0
Cluster D8-B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cluster D8-C	0	0	0	0	0	1	6	0	0	0	0	0	0	0	3	2	3	4	8	5	0	1	0	0	0	0	0	0	0	0	2	0	5	0	0	0	0	0
Cluster B3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	2	0	0	0	0	0	0	0

Fig.5

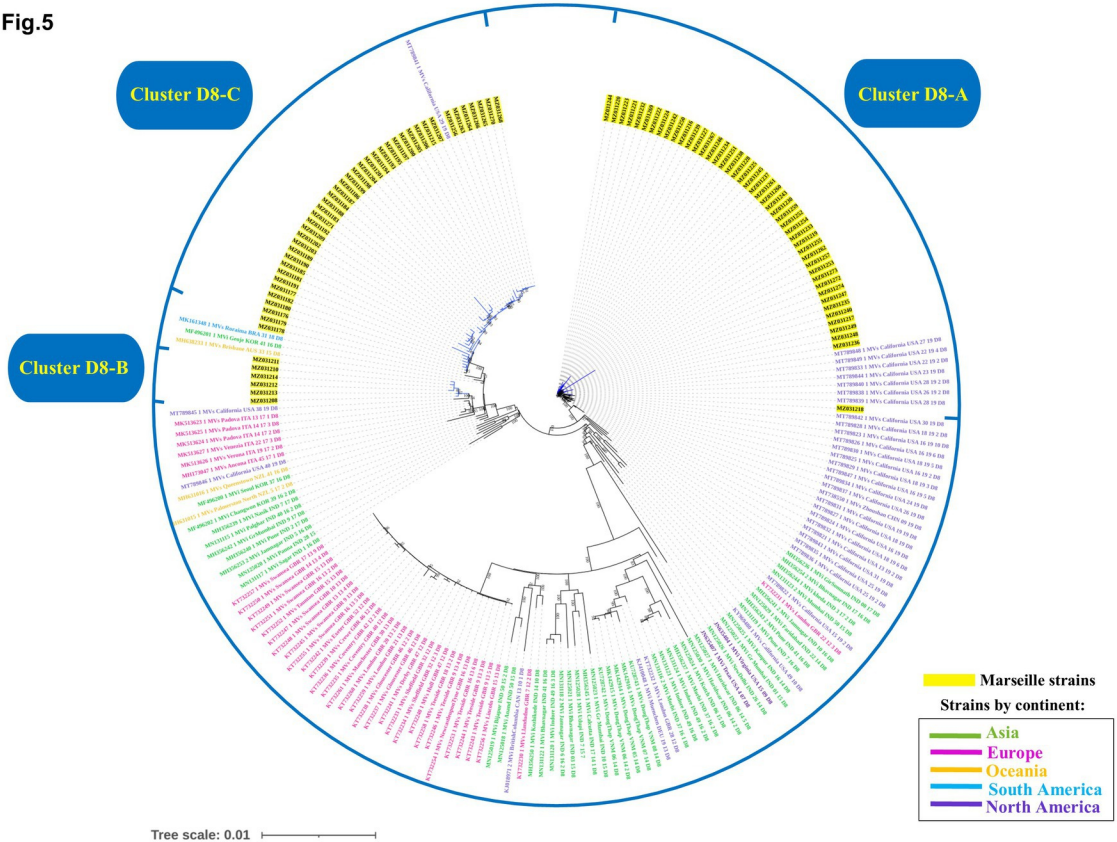
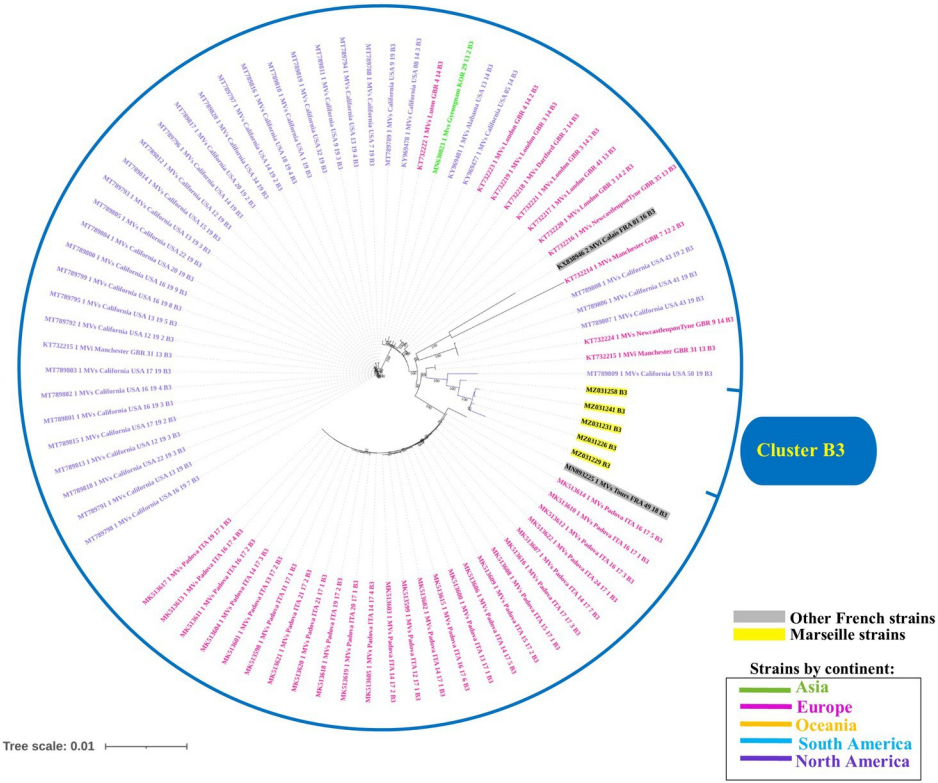

Cluster D8-A
Cluster D8-B
Cluster D8-C
Marseille strains
Strains by continent:
Asia
Europe
Oceania
South America
North America
Tree scale: 0.01

Fig.6


Cluster B3
Other French strains
Marseille strains
Strains by continent:
Asia
Europe
Oceania
South America
North America
Tree scale: 0.01