Measles Virus Genotyping and Circulating Genotypes

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Abstract: The measles virus has a single serotype although 23 genotypes have been identified by analysis of the sequences of the nucleoprotein (N) and hemagglutinin (H) genes. Infection by any genotype induces life-long immunity against all genotypes. No genotype has been associated with greater virulence or persistence. Some genotypes may be associated with specific geographic regions, although the majority cause outbreaks and sporadic cases in any country. Therefore, knowledge of the circulation of genotypes in the different World Health Organization Regions is important to enable not only the follow-up of each case of measles but also the evaluation of surveillance systems designed to achieve the elimination of measles. The presence of various genotypes in the same country in a short period of time is one of the indicators of elimination defined by the World Health Organization. Therefore, detailed study and characterization of each case of measles found in a region, the search for its origin and the evolution of its variability over time is fundamental for the elimination plans established in all WHO Regions. This work reviews which genotypes have been detected, especially in Spain.

Keywords: Measles, genotype, molecular epidemiology, measles characterization, polymerase chain reaction, Spain, humans.

INTRODUCTION

Measles is a major cause of infant mortality worldwide, mainly in developing countries, even though mortality decreased by 74% between 2000 and 2007, i.e., from 757,000 estimated deaths to 197,000. The largest percentage reduction by region was in the Eastern Mediterranean (90%) and African (89%) regions of the World Health Organization (WHO), accounting for 16% and 63% of the global reduction, respectively [1, 2].

As the incidence of measles and rubella is low in many developed countries as well as in countries with advanced control or elimination programmes, the positive predictive value for the clinical examination of cases is also low. Therefore, surveillance of individual cases is necessary and is based on laboratory diagnosis using IgM detection together with genotyping of the circulating virus strains [3].

Genetic characterization of the wild measles virus (MV) has enabled 23 genotypes to be identified, although the virus is considered to have only one serotype, since antibodies generated after infection by a specific genotype neutralize *in vitro* and protect against posterior infections by all other genotypes. None of the 23 genotypes is associated with differences in disease severity, in the probability of severe sequelae such as subacute sclerosing panencephalitis (SSPE) or inclusion body encephalitis, or variability in the sensitivity of laboratory diagnosis.

However, specific genotypes have a specific geographic distribution, and this may be useful epidemiologically for characterizing outbreaks and for designing epidemiological circulation models [4].

The first instructions for the development of a uniform nomenclature of wild MV strains were published by the WHO in 1998 [5] and updated in 2001 [6] and 2003 [7]. The genotypes defined, together with their reference sequences published in the GenBank database (http://www.ncbi.nlm.nih.gov) are shown in Table **1** [8].

The WHO recommends that the 450 nucleotides coding for the 150 amino acids of the COOH terminal of the N nucleoprotein are the minimum sequence data required for genotyping a measles virus isolate or clinical specimen. Complete H (hemagglutinin) gene sequences should be obtained from representative strains or when a new genotype is suspected [8].

The genotype of a strain is assigned by computerized comparison with the closest reference sequence, within minimum limits of similarity. If the genotype cannot be suitably identified with any known sequence, the WHO also provides guidelines for the description of a new genotype [9].

A genotype is considered endemic in a specific region if it is found for a long, more or less continuous period, whereas if different genotypes associated with limited outbreaks and/or sporadic cases are found, they probably result

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Table 1. Measles Virus Reference Genotypes Name of Strain and Access Number of GenBank Data Base for N and H Genes

Genotype	Activity	Reference Strain	Access GenBank H Gene	Access GenBank N Gene
A	Active	Edmonston-wt. USA/54	U03669	U01987
B1	Inactive	Yaounde. CAE/12.83 "Y-14"	AF079552	U01998
B2	Active	Libreville. GAB/84 "R-96"	AF079551	U01994
B3	Active	New York. USA/94 Ibadan. NIE/97/1	L46752 AJ239133	L46753 AJ232203
C1	Active	Tokyo. JPN/84/K	AY047365	AY043459
C2	Active	Maryland. USA/77 "JM" Erlangen. DEU/90 "WTF"	M81898 Z80808	M89921 X84872
D1	Inactive	Bristol. UNK/74 (MVP)	Z80805	D01005
D2	Active	Johannesburg. SOA/88/1	AF085198	U64582
D3	Active	Illinois. USA/89/1 "Chicago-1"	M81895	U01977
D4	Active	Montreal. CAN/89	AF079554	U01976
D5	Active	Palau. BLA/93 Bangkok. THA/93/1	L46757 AF009575	L46758 AF079555
D6	Active	New Jersey. USA/94/1	L46749	L46750
D7	Active	Victoria. AUS/16.85 Illinois. USA/50.99	AF247202 AY043461	AF243450 AY037020
D8	Active	Manchester. UNK/30.94	U29285	AF280803
D9	Active	Victoria. AUS/12.99	AY127853	AF481485
D10	Active	Kampala. UGA/51.00/1	AY923213	AY923185
E	Inactive	Goettingen. DEU/71"Braxator"	Z80797	X84879
F	Inactive	MVs/Madrid. SPA/94 SSPE	Z80830	X84865
G1	Inactive	Berkeley. USA/83	AF079553	U01974
G2	Active	Amsterdam. NET/49.97	AF171231	AF171232
G3	Active	Gresik. INO/17.02	AY184218	AY184217
H1	Active	Hunan. CHN/93/7	AF045201	AF045212
H2	Active	Beijing. CHN/94/1	AF045203	AF045217

from distinct imports rather than circulating endemic viruses [10]. However, in countries with sustained endemic circulation, many lineages of the same genotype coexist, whereas in epidemic outbreaks, diversity within the same genotype decreases [11].

METHODS OF VIRUS CHARACTERIZATION

In order to sequence the 450 nucleotides of the hypervariable region of the N gene or the complete H gene, one or more genomic fragments are amplified by RT-PCR (retrotranscriptase-polymerase chain reaction), either on a strain isolated from cell lines or directly from the clinical specimen. Evidently, obtaining isolates, besides being less sensitive than PCR on specimens, means more time is needed for obtaining the strain. The best clinical samples are from pharyngeal exudates and urine, since the viral genomes are only detectable in serum by PCR for a very short time, and the yield is very low. However this method should be tried in cases for which only sera are available. Sequencing the fragment obtained by PCR enables comparison of the sequence by phylogenetic analysis programmes, using the WHO reference strains (Table **1**). Once the genotype is known, comparison with the other sequences of the same genotype available in public databases enables the geographic localization of the outbreak and the follow-up of transmission within an outbreak or even between outbreaks or cases in different countries, since some genetic variability between strains of the same genotype may be observed. In countries without circulation of wild virus, where there is no variation in the carboxyl terminal of the N gene within an outbreak, differences in genes such as the phosphoprotein

gene or the H gene [12], could enable determination of whether one or more imports of the same genotype are occurring, facilitating more accurate surveillance.

Once a correct fragment is obtained, it is purified by manual methods or commercial kits. The sequencing reaction is carried out in both strands of DNA, and the nucleotide sequence is then determined by an automatic sequencer.

Programmes such as Clustal X [13], MegAlign (DNASTar, Inc.Wisconsin, USA) or BioEdit [14] are usually used to align or compare the test sequence with reference sequences; programmes like Mega v3.1 [15], Phylip (Felsenstein, J.; Washington, USA), MrBayes (Huelsenbeck, J.P., California; Ronquist, F., Florida; USA) are used to obtain the phylogenetic trees required to assign the genotype.

GLOBAL MOLECULAR EPIDEMIOLOGY OF MEASLES

Although complete MV surveillance is difficult, the continuous global flow of new reports provides an approximation of the circulation or appearance of each genotype in different WHO Regions. Virological surveillance data, when contrasted with standard epidemiological data can help to document transmission chains and the classification of each case. Likewise, they also help to document the elimination of endemic transmission and therefore provide a way to measure the effectiveness of control programmes [4].

Genotypes A, C1 and D1 were detected before the introduction of the measles vaccine. Analysis of sequences obtained from SSPE cases resulting from primary infection in the 1950s and 60s permitted the detection of genotypes C1, D1, E and F. However there is no evidence that a specific genotype has a major probability of causing SSPE: rather, the results are an indication of the efforts made to study this persistent complication of MV. Retrospective analysis of the sequences of isolates carried out during the 1970s showed continuous detection of genotypes C1 and D1 and the first detections of genotypes C2, D2, D4, and F. The remainder of the 23 genotypes were detected between 1980 and 2000; however some (B1, D1, F and G1) have not been detected in the last 15 years and are therefore considered inactive [8]. Genotype A was found in acute cases of measles in the USA [16] and South America [17], China [18], Japan [17], Russia [19], Finland [17] and the United Kingdom [20] from the 1950s to the 1990s. Current vaccines are derived from old strains of this genotype. However, the detection of genotype A in association with acute cases of measles would require detailed study, including more information on the genome detected in both specimens and isolates, in order to safely identify the vaccine-induced virus [11].

Genotype B2, previously considered inactive, has recently been detected in South Africa (2002), Angola (2003) and the Democratic Republic of the Congo (2005) [21]. B3 is the endemic genotype in West and Central Africa [22] and has been imported to various European countries in recent years [22, 23].

Genotype C1 was found in Northern Ireland, Japan, the USA, Spain and Germany in cases of SSPE [11] and in Canada [24], Japan [25] and Germany [26] in acute cases of measles. In Argentina the last outbreak caused by genotype C1 occurred at the beginning of the 1990s [27]. Genotype C2 has circulated around Europe, where it has remained endemic in different countries, but has not caused outbreaks since 2004. In addition, between 1995 and 1997, exports to Canada from France and Germany [24] and to the USA from Italy, Austria, Greece and Germany, were detected [17] and the genotype was also identified in Australia in 1990 and 1991 [28] and Morocco in 1998 and 1999 [29].

Genotype D1 was endemic in Australia in the prevaccination era [28] and was also detected in the United Kingdom in the pre-vaccine era [30] but has not been found since 1986. Genotype D2 was endemic in Southern Africa from the end of the 1970s until 2000 [31, 32] and also caused a large outbreak in Ireland in 1999-2000 [33]. Genotype D3 is endemic in Papua New Guinea [34] and, possibly, the Philippines, given the imports to the USA associated with the Philippines [11]. Genotype D4 is widely distributed and has been associated with multiple outbreaks in the Indian subcontinent and East and Southern Africa and an outbreak in Quebec (Canada) in 1989. Genotype D4 has recently been found in several European countries, including Germany (2005 and 2006), Croatia (2003-2004), Denmark (2006), France (2006), Italy (2006), Romania (2004-2007) and Russia (2000-2006) among others [23], and in Asian countries like Syria and Iran in 2003 [35], and Indian Ocean Islands such as Mayotte (2005-2006) and the Seychelles (2006) [36]. Genotypes D2 and D4 co-circulated in Southern Africa from the beginning of the 1970s until the end of the 1990s [11]. Genotype D5 is endemic in Cambodia [37]. Genotype D6 has spread widely in Europe and may have been endemic, together with genotype C2, since the 1990s. In 2005-2006, it was detected in 17 European countries, mainly with respect to a large outbreak in the Ukraine. Currently, genotype D6 is endemic in Turkey and the Russian Federation [23]. Genotype D7 circulated in the UK and Australia during the 1980s, and more recently has been found in several European countries, although it seems to be missing from Europe since 2004. It has recently been detected in India[38]. Genotype D8 seems to co-circulate with genotype D4 in the Indian subcontinent [39] and Ethiopia [40]. Genotype D9, described for the first time after its importation to Australia from Indonesia (Bali) in 1999, was isolated during the outbreak in Colombia and Venezuela in 2000-2001 and was associated with an outbreak in Japan in 2004 [11]. Genotype D10 was described for the first time in outbreaks in Uganda in 2000-2002 [31].

Group G consists of three genotypes, of which the original, G1, has not been detected since 1983; the other two (G2 and G3) are associated with transmission chains and imports from Indonesia and Malaysia [41].

Group H contains two genotypes that predominate in Asia. Genotype H1 is endemic in China and is divided into two clusters that circulate throughout the whole country [42]; it was also detected in the epidemic in Korea in 2000-2001 [43] and, since 2000, has been the predominant genotype in Japan [44]. Its circulation in Mongolia has also been verified. Genotype H2, first described in China, was recently associated with imports from Vietnam [45, 46].

Therefore, some MV genotypes are associated with a specific geographic region while others are more widely dis-

tributed. Group B predominates in Sub-Saharan and Central Africa, group G in Southeast Asia, and group H in China and Southeast Asia. Group D, however, is more widely distributed and is found in East Africa, parts of Europe and the Indian subcontinent [11].

MOLECULAR EPIDEMIOLOGY OF MEASLES IN EUROPE

In 2002, the WHO European Region designed and introduced a strategic plan for combating measles and congenital rubella syndrome (CRS), which proposes interruption of the transmission of the wild MV and a reduction in the incidence of CRS to < 1/100,000 live births by 2010. In 2004, the national heads of vaccination programmes of the European Region and the WHO European Technical Advisory Group of Experts on Immunization (ETAGE) reviewed the objectives of the plan and recommended the inclusion of rubella elimination in the strategy [47].

In the years before general use of the vaccine and up to 2005, there was no joint information on the genotypes circulating in Europe that could associate the appearance of the same genotype in different European Region countries. The evidence for this period allows few conclusions to be drawn. Between the 1960s and end of the 1990s, genotype A was detected in the former Czechoslovakia, Finland, Russia and Denmark. In 2001-2002, genotype A was detected in Belarus. Since all current vaccines belong to this genotype, after the introduction of the vaccine, the complete sequence of the H gene and/or the vaccination history must be studied to determine whether these cases are due to the wild virus or are vaccine induced.

During the 1990s, genotypes C2 and D6 were apparently the main genotypes detected throughout Europe and were endemic in some countries including Germany, at least until 2000, when a sudden replacement by genotype D7 occurred [48]. In the 1990s, genotype C2 was also detected in the Czech Republic (1992) [19], Denmark (1997) [49], Luxembourg and Holland during 1991-1994 [50], the United Kingdom in 1992-1995 [30] and Spain between 1992 and 1994 [51]. Genotype D6 was detected in the United Kingdom (1992-1995) [30], Russia (1997) and Poland (1998) [19], Turkey (1998) [52], Italy and Luxembourg (1996-1997) [50], Denmark (1998) [49] and Spain (1994-1997) [51] in the 1990s.

Other genotypes detected before 2005 were D7, which was endemic in Europe at the beginning of the second millennium in countries like Germany (2000-2001) [53], Belarus (2003) [54], Spain (2001-2003) [55], France [56] and Italy (2002) [57], and was last detected in 2004. Genotype D4 was detected in Russia (2003) [45], Croatia in an outbreak in 2003-2004 [58], Germany in 2000 [48] and Denmark (1998) [49]. Genotype H1 was detected in Russia in 2000 [45] and Germany in 2001 [53]. Genotype D8 was found in Switzerland in 2003 [59]; genotype B3 in Germany (2000) [48] and Spain (2003) [55]. Genotype D5 was observed in Switzerland in 2003 [59] and Germany in 2002 [53]. Genotype G2 was found in Germany (2001) [48], genotype D2 in an imported outbreak in Ireland in 2000 [33], and, finally, genotype D3 was detected in Denmark in 1997 [49].

The first joint European study on the circulation of genotypes in the period 2005-2006 [23] identified 9 MV genotypes in the WHO European Region. The largest outbreaks were associated with genotypes D4, D6 and B3, and the remaining outbreaks and sporadic cases were caused by genotypes B2, D5, D8, D9, G2 and H1. During 2005-2006, genotype D6 was detected in 17 of the 53 European Region countries. The diversity of these sequences is comparatively low, with a maximum genetic distance of 7 nucleotides in the hypervariable region of gene N. Two main variants, D6-2000 and D6-2005, with a single nucleotide mutation, gave rise to most cases of this genotype.

The D6-2000 variant was located predominantly in the Russian Federation during 2005 and the beginning of 2006 and also in Kazakhstan and Uzbekistan in 2006. In addition, it caused outbreaks in Germany (Bavaria, March 2005-July 2005) and Greece (September 2005-May 2006) and sporadic cases in Denmark and Israel in 2005 and Switzerland in 2006.

The D6-2005 variant caused an outbreak of more than 46,000 cases in the Ukraine between the last quarter of 2005 and October 2006. It was first identified in Russia in the last quarter of 2005 and was also detected in Azerbaijan during the first quarter of 2006. Multiple imports from the Ukraine gave rise to small outbreaks and sporadic cases in Belarus between January and September 2006, a small outbreak in Estonia in March 2006, and two sporadic cases in Latvia and Bulgaria, respectively, in April and July 2006. In Germany, this Ukrainian variant caused a large outbreak in North Rhine-Westphalia and a small outbreak in Berlin. In the United Kingdom, an outbreak of D6-2005 was associated with an import from Italy, where the variant had not been detected.

Apart from these two variants, cases due to variants other than D6 have occurred in Greece, Germany, Luxembourg and Spain (imported from Germany).

Genotype D4 is widely distributed in all continents. As mentioned, it is still endemic in the Indian subcontinent and Southern and East Africa and, before 2005, it was repeatedly identified in the WHO Eastern Mediterranean Region and in outbreaks and sporadic cases in Germany, Turkey, Spain, the United Kingdom, Croatia and Russia. In 2005 and 2006, 4 distinct groups were identified in Europe.

Group 1 includes a large outbreak in Romania with more than 8500 cases that began in December 2004 and continued until the beginning of 2007. The outbreak began in the Roma and Sinti communities before extending to the general public. Sequences that differ by ≤ 2 nucleotides were also detected in Bosnia-Herzegovina, France, Germany, Italy, Portugal, Serbia, Spain, Switzerland and the United Kingdom between 2005 and the beginning of 2007. The source of the D4 genotype in Romania could not be identified, but the homogeneity of the sequences and the large number of susceptible people indicate that it was imported.

Group 2 includes sequences found in the United Kingdom and Spain and present only one or two mutations compared with sequences from Kenya from 2002 and Ethiopia from 2003, which suggests an origin in East Africa.

Group 3 includes sequences found in outbreaks and sporadic cases in the United Kingdom during 2005-2006, and in Greece, Albania and Denmark. The large variety of sequences found in this group suggests multiples origins, although three strains from the United Kingdom and the strains from Denmark were epidemiologically-linked with Pakistan. Curiously, two genotypes, D4 and D6, were identified during what seemed to be a single outbreak in the south of Greece (January-August 2006) [60].

A group of sequences found in Germany and Denmark between February and April 2006 in the form of different outbreaks and sporadic cases form a fourth group that cannot be epidemiologically linked. The Danish virus was imported from the Lebanon, which agrees with isolates of a similar sequence in Israel in 2004. Phylogenetic analysis shows very close similarities (1-2 nucleotides difference) with sequences from Ethiopia in 2003 and Sudan in 2004. Another variant, which was imported from India, was detected in Tuscany (Italy) in January-May 2006. This virus differed by only 3 nucleotides from that found in Poland between January and May 2006, which does not concord with the epidemiological link which was first established with the Ukraine.

Genotype B3 was reported sporadically in Europe before 2005, including an outbreak in Spain in 2003 imported from Algeria and two sporadic cases in the east of Germany in 2000. However, in 2005-2006, genotype B3 was detected in eight European countries in association with outbreaks of different sizes, with a maximum genetic distance of 13 nucleotides between the sequences analyzed. A sporadic case in Holland in a person in contact with a Kenyan patient at a USA airport was detected. Outbreaks of unknown origin were also reported in Germany (January-April 2006) and the United Kingdom (June 2006), although they are suspected to have been caused by transmission within Europe. The same variant that caused the UK outbreak was imported to Spain giving rise to two outbreaks. A second variant of genotype B3, of unknown origin, was detected in Denmark, Sweden and Spain: the nearest non European sequence was detected in Nigeria in 2004. A third variant was detected in Albania and Italy. In a three-week period in 2005, two significantly-different strains were found in France and were grouped with viruses from Cameroun and Equatorial Guinea and the Democratic Republic of the Congo, respectively. A sporadic case of B3 was also detected in Switzerland in 2006. The genetic distances between all these variants suggest multiple independent imports, probably from Sub-Saharan Africa.

In summary, during 2005-2006, nine of the 17 active genotypes were found in different outbreaks and sporadic cases in the WHO European Region. The main epidemics were caused by distinct variants of genotypes D4, D6 and B3. The largest outbreaks occurred in the Ukraine (D6, > 46,000 cases), Romania (D4, >8,500 cases), Germany (D6, ≈1,700 cases) and Russia (D6, >1,100 cases). Outbreaks with between 100 and 500 cases occurred in the United Kingdom (B3), Spain (B3 and D4), Germany (D4, D6 and B3), Italy (D4), Belarus (D6) and Greece (D6, D4). In addition, smaller outbreaks and sporadic cases were caused by genotypes B2, D5, D8, D9, H1 and G3 [23].

Notably, in 2008, genotype D4 was declared as endemic in the United Kingdom, due to the continued presence of many people still susceptible to MV in Europe [61].

During 2007 the following genotypes were detected: B3 in Italy [62]; D4 in Belgium [63], Norway [64], Ireland [65] and the United Kingdom [66]; D5 in Switzerland [67]; D5 in Germany [68] and Russia [69]; D6 in Poland [70] and Russia [69]; D8 and H1 in Russia [69].

In 2008, B3 was detected in Denmark [71]; D4 in Italy [72], France [73], Germany [68], Denmark [74], Sweden [75] and Gibraltar [76]; D5 in Austria [77], Germany [78] and France [73], all of which were imported from Switzerland; D8 was detected in Holland [79] and France [80].

MOLECULAR EPIDEMIOLOGY OF MEASLES IN SPAIN

The first published sequences of MV in Spain were in patients with SSPE who had suffered measles in the 1960s, and belonged to genotype F, which is inactive today. It is thought that from approximately 1970 to 1979, the circulating genotype was C1. Later, genotype C2 predominated in 1992 and 1993 but was replaced by D6 in 1998, which circulated in Spain from the autumn of 1993 to 1997 [51]. This pattern is typical of countries with endemic circulation of MV and a high incidence of measles. After the introduction of vaccination and the gradual increase in vaccination coverages, the number of cases fell considerably to reach an incidence of 0.16 per 100,000 inhabitants in 2002 [81]. As a consequence, the circulation patterns of the virus changed, as observed after the introduction of the Spanish Measles Elimination Plan in 2001.

From 2001 to 2003, (Table **2**) genotype D7, which circulated in the rest of Europe at this time, was detected in both outbreaks and sporadic cases. In this period, other genotypes were detected in sporadic cases of imported or unknown origin, including B3, C2, D3, D4 and H1. In 2003, small outbreaks due to genotype D8 occurred in Valencia and to C2 in Castile-La Mancha. However, the most significant outbreak in this period was due to genotype B3 and occurred in Almeria province (Andalusia), with a large number of cases: this helped raise the national incidence to 0.62 per 100,000 inhabitants in 2003. In this outbreak, genotyping of the index case was carried out on a serum specimen preserved by a hospital from a patient admitted for suspected measles some weeks before who presented the same variant of genotype B3 as the patients involved in the outbreak. The index case was a sailor who had arrived in Almeria from an Algerian port some days before hospital admission.

In 2004 and 2005, measles incidence in Spain reached a historical low at 0.06 and 0.05 per 100,000 inhabitants, respectively [81]. Sporadic cases due to genotypes A, C2, D3, D4 and H1 occurred during both years (Table **2**). In 2004 one outbreak due to genotype D4 occurred in the Balearic Islands (of unknown origin), and another due to genotype D5 in Barcelona (imported from Thailand). In 2005, two small outbreaks occurred in Andalusia due to genotype D8 of unknown origin, and in Catalonia due to genotype D4 which was imported from Romania where, as already mentioned,

Table 2. Genotypes Detected in Spain between 2001 and 2008 Outbreaks in Red Italics and Sporadic Cases in Blue

AR.	2001	2002	2003	2004	2005	2006	2007	2008
Madrid	B3(GEc)	C2(Unkn)	C2(Unkn) *D7(Unkn)*	C2 (Unkn)	D4(GBR)	*B3(GBR)* B3(ETI, MAR) *D6(UKR)* A		*D4(Cádiz)* *B3 (Unkn)* D5(Unkn)
Balearic Islands	*D7(Unkn)* A H1(CHI)			*D4(Unkn)*				
C. Valencia		*D7(RUM)* D4(UKR)	*D8(Unkn)* D7(Unkn)	C2(Unkn)		*B3(Mad)* D6(UKR) *D4(Unkn)*		A
Extremadura		A		H1(CHI)				
Andalusia			*B3(ARG)* A C2(MAR) D7(Unkn)	A	*D8(Unkn)*	B3(Unkn)		*D4(Unkn)* D9(Unkn) A
Canary I.		D7(GER)		C2(Unkn)	H1(Unkn)	*B3(GBR)* *D6(GER)*		
Catalonia			C2 (Unkn) D3(Unkn)	C2(Unkn) *D5(THAI, ECU)*	*D4(RUM)*	*D4(ITA)* *D4(RUM)*	*D4 (ITA)*	
Cantabria				C2(Unkn)		*B3(Mad)*		
Murcia			*B3(Alm)*					
Castile-La Mancha			*C2(Unkn)*					
La Rioja						*D6(Unkn)*		
Castile & León							*D4(Bcn)*	
Ceuta								*D4(Unkn)*

* Unkn=Unknown; AR=Autonomic Region. GEc: Equatorial Guinea. CHI: China. RUM: Romania. UKR: Ukraine. GER: Germany. MAR: Morocco. ARG: Algeria. Alm: Almería. THAI: Thailand. ECU: Ecuador. GBR: Great Britain. ETI: Ethiopia. ITA: Italy. Mad: Madrid. Bcn: Barcelona.

this genotype caused an enormous outbreak from December 2004 to the beginning of 2007.

In 2006, several outbreaks occurred, two of which had more than 100 cases, meaning the incidence rose to 0.83 per 100,000 inhabitants [81]. At the beginning of 2006, an outbreak was detected in Logroño (La Rioja) which had begun in December 2005. The outbreak had 18 confirmed cases and was caused by the D6 genotype although no epidemiological link could be established [82]. However, comparison of the sequences indicates a close similarity to the main sequence of D6 that circulated in the Ukraine during the last quarter of 2005 until October 2006, and which was also exported to other European countries. The second large outbreak, with 174 confirmed cases, began at the beginning of 2006 in Madrid and was due to genotype B3 [83]. The index case came from the United Kingdom, where there was also an outbreak whose nucleotide sequences were similar to those found in Madrid. This outbreak caused some cases in Valencia and one case in Cantabria in a Venezuelan tourist who later exported it to his country. In Las Palmas of Gran Canaria (Canary Islands), another outbreak due to genotype B3 with 13 confirmed cases occurred between January and March. The index case had travelled to Zurich where they were in contact with an infected person from the United Kingdom. The sequences were similar to those of Madrid and the United Kingdom circulating at the same time. In Santa Cruz de Tenerife (Canary Islands), nine cases were reported between April and June, of which 3 were German tourists in whom a strain of the D6 genotype was detected. In Catalonia, two different outbreaks due to genotype D4 occurred, one at the beginning of the year with 3 confirmed cases, which was imported from Romania. The other, with 381 confirmed cases between 2006 and 2007, originated in a patient who returned from Italy and infected several families [84]. The nucleotide sequence of this second outbreak was similar to that which caused more than 8500 cases in Romania between December 2004 and the beginning of 2007 and that was also detected in other European countries. In 2006, sporadic cases occurred in Madrid due to genotype B3 (originating in Ethiopia), in Madrid and Valencia due to genotype D6 (with

a nucleotide sequence similar to that circulating in the Ukraine), and a case of genotype A (postvaccination) occurred in Madrid.

In 2008 an outbreak due to genotype D4 with 183 cases occurred, mostly in Algeciras (Andalusia); although there were also a few cases in Huelva, Malaga and Ceuta, all in Andalusia. In May, an outbreak with 11 cases was detected in Madrid; two cases had the same genotype and sequence as the cases in Algeciras. Likewise, an outbreak caused by genotype B3 produced 19 cases in Madrid: the origin was Equatorial Guinea. Two sporadic cases occurred due to genotype D4, with an origin in the United Kingdom, and to D5 and D9, of unknown origin. Two postvaccination cases were detected [81].

DISCUSSION

In large parts of the world, especially Africa and Asia, information on circulating genotypes is still scarce. More exhaustive investigation along the lines of research in recent years for characterizing the virus and publication of the results obtained is necessary for these regions where little or no information is currently available.

Remarkably, in spite of the apparent existence of inactive MV genotypes, detailed study of cases has shown that one of these supposedly inactive genotypes is not inactive as it has been maintained in various African countries, although this was not detected until 2002. This reaffirms the importance of characterization of the virus both in endemic regions and in countries where elimination is advanced.

In Europe there has been a considerable increase in the diversity of genotypes found in the same country, such as the United Kingdom, Germany and Spain, thanks to the increased surveillance mandated by elimination plans. This variability is due mainly to increased imports from regions where MV continues to be endemic, like Africa and Southeast Asia, and to the circulation of these imported genotypes within the European continent, as has occurred with D4 and B3. The increase in imported outbreaks affect people not vaccinated for various reasons or those who have received only a single dose.

On the other hand, genetic diversity within the same genotype, as in the case of D6 in 2005-2006, is much lower than during the 1990s and the first years of this century. This suggests that improvements in vaccination have greatly reduced the simultaneous circulation of highly divergent variants of D6 in Europe produced from the evolution of a common ancestor over time, with continuous circulation of the virus being replaced by imported outbreaks that are too short to generate diversity.

In addition, wide genetic diversity is also characteristic of multiple imports. The diversity of the sequences of B3 found in Europe during 2005-2008 suggests that each was directly or indirectly imported from sub-Sahara Africa. While some countries reported outbreaks caused by a single strain, others suffered epidemics caused by multiple imports of unrelated strains.

The cocirculation of different genotypes during what seemed a single outbreak has occurred in Greece, Italy and Germany. This emphasizes the importance of MV genotyping during the different phases of an outbreak. In addition, genotyping alone may not be sufficient to distinguish links between outbreaks caused by multiple imports. Epidemiological investigations in Belarus revealed multiple imports from the Ukraine that caused various, unrelated outbreaks and sporadic cases in which identical or very similar variants of D6 were found.

Currently, it is known that each MV genotype does not have a characteristic role in the clinical expression of MV infection, and neither are they the cause of reinfections or vaccination failures that may influence the epidemiological circulation of the virus, although this cannot be determined in detail without the availability of characterization methods. Genotyping of a sporadic case or an outbreak allows the hypothesis of its geographic origin established by epidemiological study to be confirmed or a hypothesis to be made. In addition, it allows coincident cases for which there is no known epidemiological link to be associated. Since this information may be relevant for the management of outbreaks, the process which leads to determination of the genotype from samples of the case or outbreak and to the availability of national and international information on what is circulating at the same time should be optimized in order to facilitate timely data supply to public health authorities Finally, global analysis of the available data on the circulation of genotypes in a specific territory is indicative of the epidemiological pattern of circulation. Analysis of the temporal evolution with sufficient perspective is fundamental for evaluating the impact of measures introduced to eliminate measles.

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APPENDIX

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