Rapid replacement of endemic measles virus genotypes

Sabine Santibanez, Annedore Tischer, Alla Heider, Anette Siedler and Hartmut Hengel

National Reference Centre Measles, Mumps, Rubella, Robert Koch Institute, Nordufer 20, D-13353 Berlin, Germany

Although vaccination campaigns have significantly reduced the number of measles cases worldwide, endemic transmission of measles virus (MV) continues to occur in several continents, including Europe. To obtain current information on measles incidence and molecular data on circulating MVs in Germany, a nationwide measles sentinel was established. Phylogenetic analysis based on the variable part of the N gene from 80 MVs isolated between November 1999 and October 2001 revealed the presence of at least six distinct MV genotypes: B3, C2, D4, D6, G2 and a new variant of D7. Both the incidence and the pattern of MV genotypes differed markedly between the former East and West Germany. In the eastern part, few measles cases, mainly caused by genotypes originating from other countries (B3, D4, G2), were detected. In the western and southern parts, genotypes C2, D6 and D7 were associated with endemic transmission. Surprisingly, the indigenous genotypes predominant during the 1990s – C2 and D6 – disappeared simultaneously over the period of observation coinciding with the emergence and the wide spread of D7 viruses. While the incidence of measles remained constant, all MVs isolated in 2001 were assigned to D7. We note that the haemagglutinin (H) sequence of D7 viruses shows distinct exchanges of certain amino acids in the stem and propeller domain compared to C2, D6 and the MV vaccine strains used. This raises the possibility of a selective advantage of D7 viruses transmitted in the presence of H-specific antibodies.

Introduction

Measles virus (MV), the only known human pathogen from the genus *Morbillivirus*, is an enveloped virus containing a negative sense (−)-RNA genome of 15894 nt. It should be possible to eliminate measles by vaccination since the serologically monotypic virus appears to have no animal reservoir. Vaccination campaigns have significantly reduced the number of measles cases worldwide. However, endemic transmission of MV still occurs in several continents, including Europe. Over 30 million measles cases and nearly 900,000 annual deaths have been reported in recent years (WHO/UNICEF, 2001).

In view of the goal of measles elimination, it is of great importance to assess the circulation of wild-type MVs. Genetic analysis has shown that distinct lineages of MVs exist and co-circulate (Rima *et al*., 1995; Bellini & Rota, 1998). Of the six genes constituting the MV genome, the nucleocapsid (N) and haemagglutinin (H) genes are the most variable with approximately 7% nucleotide variability between the most distantly related MVs (WHO, 2001). Based on the sequences derived from the variable part of the N gene encoding the 150 C-terminal amino acids and the H gene, MVs are divided into eight clades designated A, B, C, D, E, F, G and H, and further subdivided into 21 genotypes. These serve as the taxonomic unit for molecular epidemiological purposes (WHO, 2001).

Epidemiological data combined with genetic characteristics of the circulating MVs obtained from different regions of the world have suggested that there is a correlation between the observed pattern of MV genotypes and the achieved level of measles control. In countries that are still experiencing endemic transmission of MV, a limited number of circulating genotypes has been found (Xu *et al*., 1998; Hanses *et al*., 1999). Countries that have achieved an advanced level of measles control have, in contrast, reported the absence of MV genotypes detected in a consistent pattern that would indicate endemic transmission. Rather, a diversity of genotypes is found in these regions reflecting the multiple sources of imported MVs (Bellini & Rota, 1998).

In Germany, measles vaccination was introduced in the early 1970s. Compulsory vaccination in the former East
Germany achieved a high vaccination rate of > 95% whereas the voluntary vaccination in former West Germany resulted in a rate of only about 60–70%. Since reunification in 1990, vaccination is voluntary in the whole country. A mean vaccination rate of 90–3% (94.2% in the former East and 89.8% in the former West) has been established currently among 6-year-old children (W. Hellenbrand and others, unpublished).

Molecular analysis of MV isolates randomly selected during the 1990s identified the indigenous European genotypes, C2 and D6 (Rima et al., 1995; Jin et al., 1997; Santibanez et al., 1999; Hanses et al., 2000), as co-circulating in Germany (Rima et al., 1995; Santibanez et al., 1999). In order to obtain current and comparative information on measles incidence and MV circulation in Germany, a nationwide measles sentinel was established in 1999. This sentinel includes laboratory surveillance and facilitates for the first time an area-wide and continuous monitoring of measles in Germany. Based on the sentinel, MVs that were isolated during the period from November 1999 to October 2001 have been genetically characterized. The results show that the former East and West Germany have quite different patterns of MV genotypes. Unexpectedly, a dramatic and simultaneous decrease of the initially predominant genotypes, C2 and D6, was noted. Conversely, a hitherto undetected genotype, D7, was found to rapidly spread over West Germany.

Methods

■ Patients, specimens and virus isolation. MV samples were collected from 80 individuals between 1999 and 2001 in Germany. The age of the patients ranged from 6 months to 41 years with the majority infected cells was obtained using the Viral RNA kit (Qiagen). MV RNA was reverse-transcribed at 37 °C for 1 h followed by denaturation for 5 min at 94 °C using Moloney murine leukaemia virus reverse transcriptase (GIBCO BRL) and specific primers for the MV N and H genes, respectively. A part of the N gene that included the 456 nt coding for the carboxy terminus of the N protein (Nc region) was transcribed with primer MNS (nt 1131–1134, 5’ GCCATGGGAGTAGGATGGAAC) (nt positions according to Mori et al., 1999), and transcription of the coding region of the H gene was performed using primers MH1 (nt 7214–7233, 5’ CTCCTGCGGCGAAATATC) for fragment 1 and MH5 (nt 8193–8212, 5’ TTCCCTATCGGAGTACAGG) for fragment 2. Following reverse transcription, a 544 bp fragment of the Nc region (Nc fragment) was amplified by nested PCR using the Taq DNA polymerase kit (Invitek). First-round primers MNS5 and MNS6 (nt 1773–1754, 5’ CTGGCGGCCTGTGCGACCTG) and second-round primers MNS1 (nt 1196–1217, 5’ ATTAGGGCAAGAGTGGTAAAG) and MNS2 (nt 1739–1722, 5’ TATAAAGATCGTAGG) (Rima et al., 1995). Fragment 1 of the H gene (1034 bp) was amplified by nested PCR using first-round primers MH1 and MH2 (nt 8316–8297, 5’ AGCGGTGTCTATCACTTGATC) and second-round primers MH3 (nt 7248–7267, 5’ CTGGGTGCAAGATCATC) and MH4 (nt 8281–8262, 5’ GACCAAGATTTCATGGTGG). Fragment 2 (957 bp) was obtained with first-round primers MH5 and MH6 (nt 9249–9230, 5’ CAGATGCAGTCCAATACG) and second-round primers MH7 (nt 8217–8236, 5’ CTGTCAGTCCATGACTGCTC) and MH8 (nt 9173–9156, 5’ GGTATGCCGTATGCTGG). The PCR cycling programme consisted of 40 cycles (first round) of 1 min at 94 °C, 1 min at 54 °C and 2 min of 72 °C with final extension for 5 min at 72 °C followed by a second round of either 25 cycles (clinical material) or 17 cycles (supernatant from infected cells) of the same thermoprofile.

■ Nucleotide sequence determination. PCR products were sequenced in forward and reverse directions using a cycle sequencing reaction with an ABI Prism Big Dye Terminator cycle sequencing kit (Applied Biosystems, Perkin Elmer). The Nc fragment was sequenced using nested PCR primers MN1 and MN2. Fragments 1 and 2 of the H gene were sequenced using the nested PCR primers MH3 and MH4, and MH7 and MH8, respectively. The reaction products were analysed using a 3100 Genetic Analyser (Applied Biosystems).

■ Sequence analysis and genetic assignment. Nucleotide sequences were analysed and aligned with the Sequencer program, version 3.1 (Gene Codes Corp.). Phylogenetic analysis was done with PHYLP (Felsenstein et al., 1995). Phylogenograms were constructed using Dnafast followed by Neighbor and TreeView. Bootstrap values were calculated using SEQBOOT followed by Dnafast and Neighbor. Virus isolates and genotypes were designated according to the new official WHO nomenclature (WHO, 2001).

■ Estimation of measles incidence. Based on the number of the reported measles cases, the total per year was estimated by extrapolation. The number of reported cases per participating paediatrician per month and per region were multiplied by the total number of paediatricians in the respective region and calculated for Germany. Assumptions of incidences per 100’000 inhabitants and year were obtained using the estimated total number of measles cases per month and region.

Results

Measles sentinel

The German Measles Sentinel represents a network of 1273 participating medical doctors (80% paediatricians, 20% general practitioners) distributed over all parts of the country. From November 1999 to October 2001, 1755 suspected measles cases were reported. Investigation of 623 cases by serology and/or MV-specific PCR resulted in 343 (55%) laboratory confirmed cases of which 87% showed correspondence between serology and PCR; 9% were seronegative and PCR positive, and 4% were positive for MV-specific IgM and PCR negative. Eighty cases were selected for genotyping of MV depending on the local distribution (Fig. 1). This selection included 70 cases occurring in the western and southern parts of Germany, an area with widespread endemic measles, and an additional 10 cases from the eastern parts where the incidence of measles is very low.

Nucleotide sequence analysis of the MV N gene and assignment to genotypes

Phylogenetic analysis of the most variant part of the MV genome, the 456 nt encoding the carboxy-terminal region of
Replacement of measles virus genotypes

the N protein (Nc region), enabled the assignment of 80 MVs into six genotypes: B3, C2, D4, D6, D7 and G2 (Fig. 2). Unexpectedly, MVs obtained from 54 cases (68%) were classified as D7, forming a homogeneous genetic group as indicated by a maximal nucleotide difference of 0.7%. The D7 viruses found in Germany are closely related to WHO reference strain Illinois USA/50.99 (AY037020), which represents a recent D7 virus. The prototypic German D7 isolate Mainz.DEU/06.00, which has a nucleotide sequence identical with the MVs obtained from 40 cases, differs from strain Illinois USA/50.99 by only 0.4% at the nucleotide level, and both strains exhibit amino acid sequence identity. In contrast, the earlier D7 viruses isolated between 1985 and 1989 in Victoria/Australia (Chibo et al., 2000) are only distantly related to the D7 viruses found in Germany. Sequence comparison of the isolate Mainz.DEU/06.00 with the WHO reference strain of the earlier D7 viruses, Vic.AUS/16.85 (AF243450), revealed 4.0% nucleotide and 5.3% amino acid divergence. Four conservative and four non-conservative amino acid changes were detected between Vic.AUS/16.85 and Mainz.DEU/06.00.

Fourteen cases (18%) were associated with MVs of genotype C2. A maximal nucleotide difference of only 0.5% demonstrates the genetic homogeneity among the C2 viruses recently detected in Germany. A high degree of nucleotide sequence identity (99.7%) found between these viruses and a virus detected in 1996, isolate Stuttgart/96 (Y13824), indicates the close relationship between the recent and the previously circulating C2 viruses.

Eight cases (10%) were caused by MVs of genotype D6. Five MVs detected in the southern part of Germany differ from the three MVs detected in Berlin by up to 1.3% at the nucleotide level. The Berlin viruses found in 2000 are closely related to the D6 viruses observed in 1996 in Germany (AF474926, AF474924, AF474925; Santibanez et al., 1999), as indicated by a maximum divergence of 0.7%.

The genetic identification of MVs that have caused sporadic cases and a local restricted outbreak in the eastern part of Germany revealed the presence of further genotypes: MVs obtained from two sporadic cases observed in 2000 shared nucleotide sequence identity and were classified as genotype B3 (Gera.DEU/08.00 and Leipzig.DEU/09.00). They are most closely related to the WHO reference strain New York USA/94(B3) (L46753), imported into the USA from Kenya (Rota et al., 1996), from which they differ by 0.7% at the nucleotide level. A sporadic case that occurred in 2000 was caused by an MV assigned to genotype D4 (Cottbus.DEU/46.00). There is no close relationship to D4 viruses identified in other countries.

**Fig. 1.** Distribution of the laboratory confirmed measles cases in the federal states of Germany in 2000 and 2001. Abbreviations for the federal states of Germany are as follows: BY, Bayern; NRW, Nordrhein-Westfalen; BW, Baden-Wuerttemberg; HE, Hessen; NI, Niedersachsen; SH, Schleswig-Holstein; RP, Rheinland-Pfalz; NFS, New Federal States; BE, Berlin.
Fig. 2. Phylogenetic relationship between MVs isolated in Germany from 1999 to 2001 and the reference strains established by the WHO (WHO, 2001). The phylogenetic tree is based on a 456 nt sequence encoding the carboxy terminus of the nucleoprotein. Bootstrap values are given at the nodes (1000 replicates). The names of MVs are abbreviated: Gera/00, MVs/Gera.DEU/08.00, AF474928; Leipzig/00, MVs/Leipzig.DEU/09.00, AF474929; Muench./00, MVs/Muenchen.DEU/24.00, AF474932; Kempt./00, MVs/Kempten.DEU/23.00, AF474931; Tueb./00, MVs/Tuebingen.DEU/24.00, AF474933; Salzw./01, MVs/Salzwedel.DEU/25.01, AF474943; Kempt./00, MVs/Kempten.DEU/48.00, AF474937; Berlin/00, MVs/Berlin.DEU/47.00, AF474936; Berlin/01, MVs/Berlin.DEU/25.01, AF474942; Mainz/01, MVs/Mainz.DEU/07.01, AF474938; Mainz/00, MVs/Mainz.DEU/06.00, AF277805; Konst./01, MVs/Konstanz.DEU/15.01, AF474941; Koeln/01, MVs/Koeln.DEU/11.01, AF474939; Greifs./00, MVs/Greifswald.DEU/10.00/1, AF474930; Duess./00, MVs/Duesseldorf.DEU/10.00, AF474927; Bamb./01, MVs/Bamberg.DEU/14.01, AF474940; Kassel/00, MVs/Kassel.DEU/27.00, AF474934; Cottbus/00, MVs/Cottbus.DEU/46.00, AF474935. Reference strains and accession numbers are as follows: A, Edmonston-wt.USA/54, U01987; B1, Yaounde.CAE/12.83, U01988; B2, Libreville.GAB/84, U01994; B3-NY, New York.USA/94, L46753; B3-I, Ibadan.NIE/9711, A232203; C2-JM, Maryland.USA/77, M89921; C2-WTF, Erlangen.DEU/90, X84872; D1, Bristol.UNK/74, D01005; D2, Johannesburg.SOA/88/1, U64582; D3, Illinois.USA/89/1, U01977; D4, Montreal.CAN/89, U01976; D5-P, Palau.BLA/93, L46758; D5-B, Bangkok.THA/93/1, AFO79555; D6, New Jersey.USA/94/1, L46750; D7-V, Victoria.AUS/16.85, AF243450; D7-I, Illinois.USA/50.99, AY037020; D8, Manchester.UNK/30.94, AF280803; E, Goettingen.DEU/71, X84879; F, Madrid.SpA/94SSPE, X84865; G1, Berkeley.USA/83, U01974; G2, Amsterdam.NET/49.97, AY037020; H1, Hunan.CHN/93/7, AFO45212; H2, Beijing.CHN/94/1, AFO45217. In addition, MVs detected in 1996 are included: Rost.1/96, MVs/Rostock.DEU/34.96/1, AF474924; Rost.2/96, MVs/Rostock.DEU/34.96/2, AF474925; Berlin/96, MVs/Berlin.DEU/08.96, AF474926.
Replacement of measles virus genotypes

Fig. 3. Phylogenetic analysis of MVs isolated in Germany in 2000 and the WHO reference strains of the clades A, B, C and D according to the sequence of the entire coding region of the H gene (1854 nt). Bootstrap values are given at the nodes. The names of MVs are abbreviated: Gera/00, MV/Geno.DEU/00/08, AF480469; Tueb./00, MV/Tuebingen.DEU/24.00, AF480468; Kempt./00, MV/Kempten.DEU/23.00, AF480473; Muench./00, MV/Muenchen.DEU/24.00, AF480467; Berlin/00, MV/Berlin.DEU/47.00, AF489474; Greifs./00, MV/Greifswald.DEU/10.00/1, AF480472; Mainz/00, MV/Mainz.DEU/06.00, AF480470; Duess./00, MV/Duesseldorf.DEU/10.00, AF480471. Reference strains and accession numbers are as follows: A, Edmonston-wt. USA/54, U03669; B1, Yaounde.CAE/12.83, AF079552; B2, Libreville.GAB/84, AF079551; B3-NY, New York USA/94, L46752; B3-I, Ibadan.NIE/97/1, AY047365; C2-JM, Maryland.USA/77, M81898; C2-WTF, Erlangen.DEU/90, Z80808; C2-WTF, Erlangen.DEU/90, Z80808; C2-WTF, Erlangen.DEU/90, Z80808; D1, Bristol.UNK/74, Z80805; D2, Johannesburg.SOA/88/1, AFO85198; D3, Illinois.USA/89/1, M81895; D4, Montreal.CAN/89, AFO79554; D5-P, Palau.BLA/93, L46757; D5-B, Bangkok.THA/93/1, AFO09575; D6, New Jersey.USA/94/1, L46749; D7-V, Victoria.AUS/16.85, AF247202; D7-I, Illinois.USA/50.99, AYO43461; D8, Manchester.UNK/30.94, U29285.

since the nucleotide sequence differs by 1.3% from the most closely related strain, Pennsylvania.USA/17.97 (AY037031). A single case of a locally restricted outbreak (n = 19) in 2001 was associated with an MV classified as genotype G2 (Salzloeke.DEU/25.01). It is most closely related to strain Amsterdam.NET/49.97(G2) (AF171232), imported into the
Netherlands from Indonesia (de Swart et al., 2000) from which it differs by 0–4%.

In conclusion, the genetic assignment of the sequences of the Nc region revealed the presence of at least six MV genotypes in Germany between November 1999 and October 2001. Unexpectedly, a new variant of the D7 genotype was found to be predominant in the western and southern parts of Germany.

**Sequence comparison of the MV H gene**

The H protein is the most important target for neutralizing antibodies and is currently thought to be subject to immunological pressure (Giraudon & Wild, 1985; Rota et al., 1992). Therefore, the H gene sequence can contribute not only to assessment of the phylogenetic relations between MVs, but can also provide a basis for characterization of their antigenicity. The complete sequence of the coding region of the H gene (1854 nt) was determined for four D7 viruses, three C2 viruses, one D6 virus and one B3 virus (Fig. 3).

Nucleotide sequence identity of ≥ 99–7% found among the recent German D7 viruses documented the genetic homogeneity within this group. Sequence similarity of the H gene confirmed that the German D7 viruses are most closely related to the WHO reference strain Illinois USA/50.99 (AY043461) from which they differ by ≤ 0–3% at the nucleotide level and ≤ 0–6% at the amino acid level. In comparison to the Nc region, the H gene demonstrates an apparently closer relationship between the recent German and the previously observed Australian D7 viruses; i.e. strains Mainz.DEU/06.00 and Vic/AUS/16.85 (AF247202) diverge by 1–3% at the nucleotide level and 1% at the amino acid level.

Detailed amino acid sequence comparisons among the three genotypes that were endemic in Germany in 2000 (D7, C2 and D6) revealed exchanges of seven amino acid residues between the new genotype D7 in comparison with genotypes C2 and D6. These exchanges were found at amino acid positions 174 (T → A), 176 (T → A), 195 (R → K), 296 (L → F), 302 (G → R), 308 (I → V) and 416 (D → N) of the H protein. At these positions, the prototypic vaccine strain Edmonston is identical with both the C2 and D6 viruses. According to the structural model proposed for the morbillivirus H molecule by Langedijk et al. (1997), three exchanges (positions 174, 176 and 195) are located in the stem 2 region, while the others (296, 302, 308 and 416) reside within the β-sheets forming the propeller structure of the H protein. The substitution at amino acid 416 (D → N) resulted in an additional potential N-linked glycosylation site in the H protein of the German D7 viruses.

**Geographical and temporal distribution of MV genotypes**

The distribution of the six observed MV genotypes over the regions of Germany, indicated by a widely differing incidence of measles, demonstrates a correlation between the genotype pattern and the incidence (Fig. 4). In the southern part (Bayern, Baden-Wuerttemberg) where a high incidence was noted, genotypes C2 and D6 were predominant in 1999/2000: 14 C2 viruses, five D6 viruses and only one D7 virus were detected. In contrast, in 2001, all MVs (n = 11) were identified as genotype D7. In Nordrhein-Westfalen, located in the western part and also indicated by a high incidence, genotype D7 was exclusively observed in 2000 and 2001 (Fig. 4). In regions with a moderate and low incidence in the western part of Germany and in Berlin, genotype D7 was predominant in 2000 whereas genotypes C2 and D6 were observed only rarely. In 2001, only genotype D7 was found (Figs 4, 5).

A quite different pattern of genotypes was obtained from the eastern part of the country where the incidence of measles is very low. Almost exclusively, genotypes that were not
indigenous in Central Europe, i.e. B3, D4 and G2, were detected, while one locally restricted outbreak was caused by genotype D7.

**Discussion**

This study presents a systematic and extensive description of the molecular epidemiology of measles in Germany. For the first time, MVs were genetically characterized in the context of a nationwide measles sentinel that enabled an area-wide and a continuous monitoring of circulating MVs from November 1999 to October 2001. Phylogenetic analysis of the nucleotide sequences revealed the presence of six genotypes: B3, C2, D4, D6, D7 and G2 (Fig. 2). Genotypes C2, D6 and D7 were associated with endemic transmission of MV in the western and southern parts of Germany. A profound change in the genotype pattern was noted: C2, D6 and the newly discovered D7 were detected in the year 2000, but the only genotype identified in 2001 was D7. Genotypes B3, D4, D7 and G2 caused sporadic cases or locally restricted outbreaks in the eastern part of Germany, where the incidence of measles is < 1 case per 100000 population per year.

Prior to this study, only a few MVs collected sporadically in some parts of Germany had been analysed, revealing the circulation of MVs assigned to only two genotypes, C2 and D6 (Rima et al., 1995; Santibanez et al., 1999). Both genotypes were nearly exclusively observed in Central and Western Europe indicating indigenous circulation in Europe during the 1990s (Rima et al., 1995, 1997; Jin et al., 1997; Santibanez et al., 1999; Hanses et al., 2000).

This paper is the first report on the endemic circulation of genotype D7, which became predominant and widely distributed over the western and southern parts of Germany. From 1999 to 2001, 68% of the measles cases from all parts of Germany were caused by D7 viruses. Previously, a single case of D7 observed in 1999 in Illinois, USA, was attributed to virus importation from Europe (P. Rota, personal communication). This assumption is supported by our finding that the Illinois virus is closely related to the contemporary D7 viruses in Germany. In addition, importation of D7 viruses from Europe has been observed recently in Canada (G. Tipples, personal communication) and El Salvador (PAHO, 2001). A genetically stable virus group from Australia, circulating between 1985 and 1989 in Victoria, was first assigned to genotype D7 (Chibo et al., 2000). A high degree of divergence of 4% in the variable part of the N gene and 1-3% in the coding region of the H gene has been found between the Australian and the German D7 strains. Considering the genetic stability of MVs, it seems unlikely that an Australian D7 virus represents a progenitor of the D7 viruses circulating at present in Germany.

The epidemiological results of the measles sentinel demonstrate that Germany has distinct regions characterized by a widely differing incidence. The pattern of MV genotypes correlates with the level of incidence. In the southern part of Germany (Bayern, Baden-Wuerttemberg), a high incidence indicated an endemic circulation of MV. In the year 2000, the indigenous genotypes in the 1990s, C2 and D6, were the predominantly ones in circulation. In 2001 neither genotype was observed, and only the new genotype, D7, was present. In Nordrhein-Westfalen, a densely populated region in the
western part of Germany with a high incidence of measles, D7 viruses were already detected from the beginning of 2000, and were exclusively circulating in 2000 and 2001. It is not known which genotypes were present here before. The neighbouring Benelux countries reported observation only of genotypes C2 and D6 in 1996/97 (Haneses et al., 2000), suggesting that D7 viruses started spreading in Nordrhein-Westfalen in the late 1990s. It is tempting to speculate that the D7 genotype is now circulating in other European countries besides Germany.

The eastern part of Germany has reported a very low incidence of measles since the mid-1980s. Accordingly, only a small number of cases occurred, which were associated with certain MV genotypes, most likely imported from regions outside Europe. Two sporadic cases were caused by genotype B3, which might have been imported from sub-Saharan Africa (Bellini & Rota, 1998; Haneses B3, which might have been imported from sub-Saharan Africa outside Europe. Two sporadic cases were caused by genotype certain MV genotypes, most likely imported from regions

1990s. It is tempting to speculate that the D7 genotype is now circulating in other European countries besides Germany.

The eastern part of Germany has reported a very low incidence of measles since the mid-1980s. Accordingly, only a small number of cases occurred, which were associated with certain MV genotypes, most likely imported from regions outside Europe. Two sporadic cases were caused by genotype B3, which might have been imported from sub-Saharan Africa (Bellini & Rota, 1998; Haneses et al., 1999). Another sporadic case was associated with genotype D4. Lacking a high degree of similarity with the genotype D4 viruses identified before, the source of this MV remains unclear. MV obtained from a single case of a locally restricted outbreak was identified as genotype G2. It might have been imported from Indonesia where very similar viruses have been detected since 1997 (de Swart et al., 2000; Rota et al., 2000). Interestingly, one local restricted outbreak was caused by genotype D7. The pattern of presumably imported genotypes and their local and temporally restricted emergence indicate that there is no endemic circulation of MV in the eastern part of Germany.

Although measles is still endemic in many countries, mainly in Africa and Asia, MV circulation is not well understood. Data describing the genetic characteristics of endemically circulating MVs during the 1990s are available only from some regions, e.g. South Africa (Kreis et al., 1997), sub-Saharan Africa (Haneses et al., 1999; Truong et al., 1999), Ethiopia (Nigatu et al., 2001), China (Xu et al., 1998), Vietnam (Liffick et al., 2001) and Nepal (Truong et al., 2001). These studies have shown that the number of genotypes is restricted in regions with endemic measles. For South Africa, a change of MV genotypes that occurred in Johannesburg between the late-1980s and the mid-1990s was reported (Kreis et al., 1997). Changes of MV genotypes have also been observed in Europe; i.e. several genotype switches occurred between the late-1960s and the mid-1990s in Madrid (Rima et al., 1997). These earlier studies did not assess the MV circulation in an area-wide and continuous manner, and the data obtained were not sufficient to monitor changes of the genotype pattern within a defined time frame. Here, we have recorded the process of a simultaneous disappearance of two genotypes that were circulating endemically for a number of years and the concomitant emergence and rapid spread of a new endemic genotype. The question arises as to why C2 and D6 viruses have been rapidly replaced by D7 viruses, whereas previously both genotypes could co-circulate for a long period. It is feasible that the appearance of D7 viruses simultaneously coincided with the disappearance of C2 and D6 viruses without any mutual influence. However, we favour the idea that the D7 viruses now have a decidedly selective advantage over C2 and D6 viruses. This view is supported by the remarkably rapid spread of D7 viruses over the former Western Germany. The phylogenetic relations between the genotypes are apparently not decisive since C2 and D6 disappeared almost simultaneously even though different genetic distances to D7 are present. Among several possible reasons, e.g. a different efficiency of MV replication, one explanation could be related to the viral surface glycoprotein H. This is responsible for the attachment of the virion to its host-cell receptor (Wild & Buckland, 1995; Tatsuo et al., 2000; Schneider-Schaulies et al., 2001) and acts in conjunction with the viral fusion protein during fusion and virus entry (Wild et al., 1991; Cattaneo & Rose, 1993). MV H is the major target for virus neutralizing antibodies (Giraudon & Wild, 1985), and in this context it is noteworthy that the D7 viruses differ from the previously circulating C2 and D6 viruses by seven amino acid residues in the H protein, including an additional N-linked glycosylation site. Moreover, the Edmonton vaccine virus is identical with both the C2 and D6 viruses at these positions. Addition of a N-linked glycan at amino acid position 416 has been reported to correlate with the loss of haemagglutinating activity (Saito et al., 1995). Moreover, the point mutations found within the structure of the H protein map closely to the location of linear epitopes of antibodies (Makela et al., 1989a, b). Point mutations including changes in glycosylation are known to modify antibody binding to the influenza A virus H protein. Remarkably, antigenic drift of influenza A H3N2 viruses over the last 30 years has resulted in the selection of viruses carrying an increasing number (3 → 7) of N-linked glycosylations in the H protein (reviewed in Hay et al., 2001). Antibodies derived against the vaccine MV strains still neutralize wild-type MVs (Bellini et al., 1994; Lee et al., 2000). However, certain wild-type viruses are neutralized with significantly lower efficiency by sera from vaccinees (Klingele et al., 2000), and the more resistant viruses are not limited to a certain clade of MVs. Vaccinees who are protected against overt disease may, nevertheless, undergo asymptomatic MV infection as indicated by secondary immune responses (Pedersen et al., 1989; Vardas & Kreis, 1999). In those individuals, an MV with a lower susceptibility to pre-existing neutralizing antibodies might have a selective advantage in terms of replication and the maintenance of chains of transmission. This hypothesis awaits further evidence by testing the capacity of sera from vaccinees to neutralize MVs of different genotypes. The fact that D7 viruses could not establish endemic spread in the eastern part of Germany where the coverage of measles immunization is high suggests that vaccination is generally effective in protecting against measles disease irrespective of the MV genotype.

We are grateful to I. Deitemeier, V. Wagner and A. Wolbert for expert technical assistance, A. Mas Marques for advice on the
References


Truong, A. T., Mulders, M. N., Gautam, D. C., Ammerlaan, W., de


Received 4 March 2002; Accepted 4 July 2002