Update on the Global Distribution of Genotypes of Wild Type Measles Viruses

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Molecular characterization of measles viruses is an important component of measles surveillance because these studies enhance our ability to identify the source and transmission pathways of the virus. Molecular surveillance is most beneficial when it is possible to observe the change in virus genotypes over time in a particular region. Such information can help to document the interruption of transmission of measles virus and thus provide an important method for assessing the effectiveness of vaccination programs. It is recommended that virus surveillance be conducted during all phases of measles control and be expanded to give an accurate description of the global distribution of measles genotypes. This review provides updated information on the circulation patterns of measles genotypes and examples of the utility of virologic surveillance.

Recent international efforts to control measles infections through aggressive vaccination programs have had a great deal of success. In particular, the Pan American Health Organization (PAHO) reported record low numbers of measles cases in the Americas during 2000–2001, reflecting the overall success of measles control programs in the Western Hemisphere [1]. Indigenous transmission of measles virus has been eliminated in the United States, the most populous country in the region of the Americas, and only 3 of 41 countries in the region reported indigenous measles transmission during 2001 [1, 2]. The success of mass vaccination campaigns in southern Africa has suggested that measles elimination is possible even in developing countries with a high incidence of human immunodeficiency virus infection [3]. However, despite these successes, measles remains an endemic disease in many areas of the world, and among children, it is still the most common cause of death from a vaccine-preventable disease. The World Health Organization (WHO) estimates that ~45 million cases of measles, with nearly 800,000 fatalities, continue to occur annually [4, 5]. Measles outbreaks still occur in both developed and undeveloped countries that have not maintained adequate immunization levels.

Genetic characterization of measles viruses has proven to be a powerful adjunct to the standard epidemiologic techniques that are used to study the transmission of measles. Molecular data help confirm the sources of virus or suggest a source for “unknown source cases.” These data can also help to establish links, or lack thereof, between various cases and outbreaks. Molecular surveillance is most beneficial when it is possible to observe the change in virus genotypes over time in a particular region because this information, when analyzed in conjunction with standard epidemiologic data, has helped to document the interruption of transmission of endemic measles. Thus, molecular characterization of measles viruses has provided a valuable tool for measuring the effectiveness of measles control programs. This review summarizes the current state of knowledge regarding the global distribution of measles genotypes and describes examples of how virologic surveillance has contributed to measles control.
MOLECULAR EPIDEMIOLOGY OF MEASLES VIRUS

Molecular virology. Measles virus is an RNA virus in the Morbillivirus genus within the family Paramyxoviridae. Although other members of the genus infect various animal species, measles only infects humans and nonhuman primates. The negative-sense, single-stranded RNA genome is contained within a helical nucleocapsid in the virion. The genome consists of 15,894 nucleotides, which code for the six structural proteins (nucleoprotein [N], phosphoprotein, and matrix, fusion, hemagglutinin [H], and large protein) and two nonstructural proteins, C and V [6].

Although measles is a monotypic virus, genetic and antigenic variation has been detected in wild type viruses [7–10]. Many laboratories have conducted sequence analysis of wild type measles and demonstrated that molecular epidemiologic techniques could be used to study the transmission patterns of measles virus [11–16]. Genetic characterization of wild type measles viruses has focused on analysis of the genes coding for the H and/or N proteins, which contain up to 8% nucleotide variability. One of the most variable parts of the measles genome is the 450-nucleotide region, which codes for the COOH-terminus of N protein, where nucleotide variability can approach 12% between wild type viruses [17].

Standardization of molecular virologic surveillance methods. Before 1998, there was no uniform nomenclature or analysis protocol to describe the genetic characteristics of wild type measles viruses. In the spring of 1998, WHO held a meeting to address these issues, and the WHO recommendations from the 1998 meeting were updated in 2001 [18–20]. A uniform nomenclature for naming strains and describing genotypes was adopted, and a standard analysis protocol was established so that sequence data would be directly comparable between laboratories. WHO recommended that the 450 nucleotides that code for the COOH-terminal 150 amino acids of N are the minimum amount of sequence data required for genotyping a measles virus isolate or clinical specimen. Complete H gene sequences should be obtained from representative strains or if a new genotype is suspected. Phylogenetic analysis of the H gene sequences, which contain up to 8% nucleotide variability between strains, provides additional support for the genotype assignment while monitoring amino acid substitutions that could affect antigenicity.

For molecular epidemiologic purposes, the genotype designations are considered the operational taxonomic unit, while related genotypes are grouped by clades. WHO currently recognizes 8 clades, designated A, B, C, D, E, F, G1, and H. Within these clades, there are 20 recognized genotypes and 2 proposed new genotypes (g3 and d9). Genotypes are designated A, B1, B2, B3, C1, C2, D1, D2, D3, D4, D5, D6, D7, D8, E, F, G1, G2, H1, and H2. Some clades contain only 1 genotype and, in such cases, the genotype designation is the same as the clade name. Other clades, such as clade D, contain multiple genotypes and are designated by using the clade letter (in uppercase) and genotype number (e.g., D1, D2). For proposed genotypes, the lowercase clade letter will be used until the genotype is recognized (e.g., g3, d9). Several of the genotypes, E, F, G1, D1, appear to be extinct or inactive since representatives of these genotypes have not been isolated for at least 15 years. However, the sequences of the inactive genotypes are maintained in the set of WHO reference sequences for completeness [19].

With the exception of genotype F, all of the genotypes have an assigned reference strain chosen to represent the earliest isolation of virus from each genotype. Sequences from recent virus isolates are then compared to the set of WHO reference sequences, which are available from GenBank [19] and the WHO Strain Banks, to determine the genotype. Since the molecular surveillance for measles is still incomplete, new genotypes are being identified frequently. WHO has established guidelines based on both molecular biologic and epidemiologic criteria for the designation of new genotypes [19].

Whenever possible, attempts should be made to isolate virus by using the preferred cell line, B95a [21]. B95a cells are an Epstein-Barr virus–transformed, marmoset B lymphoblastoid cell line that is up to 10,000 times more efficient for detecting measles virus in clinical specimens than are fibroblast cell lines, such as Vero [21]. When appropriate, reverse transcriptase–polymerase chain reaction (RT-PCR) can be used to amplify measles genes directly from clinical specimens.

Countries involved in measles elimination, should collect appropriate specimens for virus isolation from every chain of transmission, while countries involved in outbreak control and mortality reduction should obtain representative specimens from measles outbreaks [22]. It is important to conduct virologic surveillance before accelerated control measures are initiated so that it will be possible to study the pattern of genotypes present both before and after vaccination campaigns. To facilitate increased measles control and possible elimination activities, WHO is developing a Global Measles Laboratory Network. This network, which is patterned after the laboratory network that is currently in place for polio surveillance, will consist of national, regional, and global laboratories. In addition to performing serologic diagnosis of measles infections, the measles laboratory network will also support improved and expanded virologic surveillance.

Mutation rates and antigenic variation. Laboratory studies have estimated that the mutation rate of measles virus is similar to those of other RNA viruses [23]. However, molecular epidemiologic studies of the measles resurgences in Brazil in 1997 [24] and in the United States in 1989–1991 [14] suggest...
that there is very little sequence variation in the N and H genes within a single chain of measles transmission. Nucleotide sequences from the N genes of viruses isolated during the large outbreak in São Paulo in 1997 were nearly identical to the sequences obtained from viruses that had spread to other states in Brazil as well as other South American countries during 1997 and 1998 [24]. The genotype D3 viruses that were isolated during the resurgence of measles in the United States during 1989–1992 showed very little sequence variability in both the H and N genes, suggesting that 1 strain of virus had seeded the entire country [14]. Even in areas with endemic transmission of virus, there appears to be very little sequence variation present in viruses isolated from the same chain of transmission. However, the sequences of viruses from measles-endemic areas show more variation within a genotype because the epidemiologic conditions favor maintenance of multiple chains of transmission [25]. Last, the genetic stability of measles field isolates was also noted by sequencing viruses from the same genotype that had been isolated several years apart [26].

The relatively recent discovery of genetic variation within wild type measles viruses has led to the suggestion that these viruses have antigenic characteristics that allow them to circulate more efficiently in the presence of vaccine-induced immunity. Although antigenic differences between measles viruses from the various genotypes have been detected by using monoclonal antibodies and polyclonal antiserum [7, 10], the wild type viruses that have the most sequence variation compared with the vaccine are neutralized by antiserum to the vaccine virus [17]. More important, measles vaccination programs, when properly administered, have been exceptionally successful when properly administered. Studies are in progress to explore the potential for biologic differences between measles viruses from different lineages [1, 3].

**Measles vaccines.** Sequence analyses have shown that all of the measles vaccine strains are representatives of genotype A [27]. This includes both vaccines derived from the original Edmonston isolate of 1954 (e.g., Moraten, Schwarz, Edmonston-Zagreb, AIK-C) as well as vaccines derived from other wild type viruses isolated during the 1950s and 1960s in China and Japan (e.g., Shanghai-191, Chanchun-47, CAM-70). While this suggests that genotype A viruses had a wide distribution in the prevaccine era, it is also possible that genotype A viruses were more frequently detected because they were easier to isolate in the cell culture systems available at the time. Genotype A viruses have been isolated from sporadic measles cases in the last 10 years, but there have been no reports that this genotype has been associated with any large outbreaks. Though it is possible that wild type genotype A viruses are still circulating, there is a strong likelihood that recently detected genotype A viruses are vaccine viruses or laboratory contaminants. Efforts are underway to attempt to identify a set of genetic markers to distinguish wild type, genotype A viruses from vaccine viruses.

A small proportion of measles vaccine recipients experience rash and fever 10–14 days following vaccination. During outbreaks, measles vaccine is distributed to help control the outbreak, and in these situations, vaccine reactions may be mistakenly classified as measles cases. Since serologic methods cannot distinguish between vaccine-induced immunity and immunity from natural disease, molecular characterization of virus isolates provides a method to confirm whether the rash and fever were caused by vaccine or wild type measles virus.

**GLOBAL DISTRIBUTION OF MEASLES GENOTYPES**

Where relatively extensive virologic surveillance has occurred, two general patterns of measles genotype distribution have been observed. In countries that still have endemic transmission of measles, most cases are caused by relatively few endemic genotypes. In countries that have eliminated measles, the small numbers of cases are caused by a number of different genotypes that reflect various imported sources of virus and suggest the lack of sustained transmission of an endemic genotype. These paradigms will be discussed in greater detail below.

**Molecular epidemiology in countries that have eliminated measles.** Endemic transmission has been eliminated in many areas of the world, including most of the countries in the Western Hemisphere. Both virologic and epidemiologic data collected in the United States between 1989 and 2000 indicated that interruption of transmission of the genotype D3 viruses that were associated with the measles resurgence of the early 1990s was achieved in 1993 and subsequently maintained [14, 28]. Analysis of viruses isolated from measles cases and outbreaks in the United States between 1994 and 2001 failed to detect ongoing transmission of an endemic genotype (figure 1). Rather, the diversity of genotypes detected in the last 7 years is indicative of multiple, imported sources of virus [28, 29]. Likewise, the diversity of genotypes detected in Australia, Canada, and the United Kingdom is similar to that observed in the United States, suggesting frequent importation of measles and lack of endemic circulation of virus [30–33].

Though virologic surveillance has improved recently in South America, there is no record of the endemic genotypes that circulated before PAHO launched its very successful measles elimination efforts in the early 1990s. However, recent molecular epidemiologic studies have demonstrated interruption of circulation of genotype D6 viruses that were responsible for the large measles outbreak in São Paulo in 1997 and subsequent outbreaks in Rio de Janeiro, Argentina, Chile, Bolivia, Haiti, and the Dominican Republic [24, 34–37]. The record low num-
Figure 1. Measles cases and measles genotypes detected in the United States, 1980–2001. D3 was the indigenous genotype during the measles resurgence between 1989 and 1991. Since 1993, a variety of genotypes were isolated, but no single genotype has been consistently detected. Genotype D3 was associated with 2 imported cases. Prior to 1988, virologic surveillance was poor; only 2 isolates were available for the years 1980–1988.

Distribution of measles genotypes in measles-endemic areas. Virus surveillance is still incomplete, and isolates have not been obtained from many parts of the world, including many areas with endemic measles. It will be important to characterize viruses from all parts of the world in the next few years in an effort to develop a complete genetic baseline before accelerated measles control programs are initiated. Table 1 and figure 2 show the measles genotypes that have been detected in areas with endemic measles or frequent measles outbreaks. Also listed in table 1 are countries that have endemic or widespread measles and have been identified as the source of importation of a particular genotype into other countries. In these latter cases, the circulation of a genotype has not been verified by virologic surveillance in the source country but was inferred on the basis of a consistent pattern of importations. For example, although genotype D3 viruses have never been isolated in the Philippines, there have been several instances of genotype D3 being detected in measles cases imported into the United States from the Philippines. In each of these cases, standard case investigation confirmed that the individuals were traveling in the Philippines during the incubation period.

Because of the relatively low vaccination coverage rates in many countries, measles continues to circulate in Western Europe. The most frequently isolated measles genotypes in Europe have been C2 and D6 [42–44], and genotype D7 [45] viruses have been shown to circulate widely in the western part of Germany. Because of the frequency of travel to and from Europe, genotypes C2, D6, and D7 are often associated with measles cases imported from Europe to other parts of the world [28, 29, 38].

Several measles genotypes have been identified in Africa. Clade B viruses are endemic in the central and western parts of sub-Saharan Africa, and recent analysis of a large number of recent measles isolates from Nigeria, Ghana, The Gambia, Cameroon, and Sudan supports the division of clade B into 3 genotypes, B1, B2, and B3 [46–49]. Genotype B3 has been divided into two clusters. Genotype B3 cluster 1 viruses have been isolated from Cameroon, Ghana, and Nigeria and from as far east as Sudan, suggesting that clade B viruses are widely distributed in sub-Saharan Africa. The circulation of genotype B3 cluster 2 viruses appears to be more limited to western Africa [50]. In contrast, genotypes D2 and D4 have been the most frequently detected genotypes in the southern and eastern parts of the African continent [51, 52]. Virus isolates from only one northern African country, Morocco, have been characterized. The Moroccan viruses were all in genotype C2, suggesting that the pattern of measles genotypes in northern Africa may be more related to the European pattern than to the pattern seen in other parts of Africa [53].

Measles is endemic on the Indian subcontinent. Genotype D4 and D8 viruses have been isolated in India and Nepal, and genotype D4 was detected in Pakistan [20, 54, 55]. Genotype D4 viruses have also been detected in measles cases imported into the United States from both Pakistan and India [28, 29].
Table 1. Current knowledge of the global distribution of wild type measles viruses.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Countries with endemic measles or frequent outbreaks or countries identified as the source of imported cases: 1995–2001</th>
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<tbody>
<tr>
<td>B3</td>
<td>Nigeria, Ghana, Kenya, Sudan, Congo, Democratic Republic of Congo, The Gambia, Cameroon</td>
</tr>
<tr>
<td>C2</td>
<td>Morocco, Czech Republic, Germany, Denmark, Luxembourg, Spain</td>
</tr>
<tr>
<td>D2</td>
<td>South Africa, Zambia</td>
</tr>
<tr>
<td>D3</td>
<td>Japan, Taiwan, Philippines&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>D4</td>
<td>India, Pakistan, Iran, Kenya, Zimbabwe, Namibia, South Africa, Russia, Ethiopia</td>
</tr>
<tr>
<td>D5</td>
<td>Japan, Thailand</td>
</tr>
<tr>
<td>D6</td>
<td>Russia, Brazil, Argentina, Bolivia, Italy&lt;sup&gt;a&lt;/sup&gt;, Turkey, Germany, Poland, Dominican Republic, Luxembourg, Spain</td>
</tr>
<tr>
<td>D7</td>
<td>Germany, Spain</td>
</tr>
<tr>
<td>D8</td>
<td>Ethiopia, Nepal, India</td>
</tr>
<tr>
<td>d9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Indonesia, Venezuela</td>
</tr>
<tr>
<td>G2</td>
<td>Indonesia, Malaysia</td>
</tr>
<tr>
<td>g3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>East Timor, Indonesia</td>
</tr>
<tr>
<td>H1</td>
<td>China, Korea</td>
</tr>
<tr>
<td>H2</td>
<td>Vietnam</td>
</tr>
</tbody>
</table>

<sup>a</sup> Identified as source of imported virus only.
<sup>b</sup> Proposed new genotype, pending isolation of reference strain.

Extensive virologic surveillance in Japan has demonstrated that genotypes D3 and D5 have been co-circulating in Japan for at least 10 years [56–58]. Mostly genotype D5 viruses have been associated with many measles cases imported from Japan [28, 29]. These data provide evidence that multiple genotypes of measles can co-circulate if there are sufficient numbers of susceptible individuals in the population to sustain transmission. The data also suggest that measles genotypes do not displace one another as do new variants of influenza virus.

Elsewhere in Asia, sequence analysis of wild type measles viruses isolated in several provinces in the People’s Republic of China show widespread distribution of viruses in genotype H1 [17]. Viruses that were indistinguishable from the Chinese genotype H1 viruses were isolated during the outbreak of measles in Korea during 2000–2001 [59]. Virologic surveillance was not conducted in South Korea before the outbreak, so it is not possible to determine if H1 viruses were endemic to South Korea or were recently introduced by importation from China. Wild type measles viruses in Vietnam are also classified as clade H, but they are sufficiently different from the Chinese viruses to be designated as a separate genotype, H2 [25]. It is interesting to note that wild type measles viruses isolated in Thailand are...
in genotype D5 [20, 28] and more closely related to viruses circulating in Japan than to viruses circulating in other parts of Asia.

Until recently, the only measles viruses representing clade G had been isolated in 1983, and this clade was considered to be inactive. In 1997, a virus belonging to clade G was isolated from an Indonesian child who was being treated at a Dutch hospital. The H and N sequences of this virus were sufficiently different from the reference strain for it to be considered a new genotype (G2) within clade G [60]. Viruses belonging to genotype G2 have recently been isolated in Indonesia and Malaysia [61]. In addition, viruses from two proposed new genotypes have been isolated in Indonesia and East Timor. RT-PCR and sequence analyses of clinical specimens obtained from measles cases imported into Australia indicated that viruses circulating in East Timor represent a proposed third genotype within clade G, g3 [33, 62]. Viruses in genotype G2 and proposed genotypes g3 and d9 have been recently detected as part of virologic surveillance activities in Indonesia (Rota PA, Bellini WJ, unpublished data).

**SUMMARY**

The recent identification of new measles genotypes and the rate at which these new genotypes have been found suggest that our understanding of the extent of genetic heterogeneity present among wild-type measles viruses is still far from complete. Virus isolates have not been obtained from many parts of the world that still have endemic measles and, clearly, the quality of virologic surveillance needs to be improved in all areas. The recent standardization of the molecular techniques and the continuing expansion of the WHO Global Measles Laboratory Network will facilitate rapid advances in virologic surveillance activities. An important activity of the laboratory network will be to develop methods to improve measles surveillance in remote areas. In particular, establishing protocols for collection of specimens that can be stored and transported at ambient temperature, such as the dried blood spots on filter paper [63–65], will facilitate expansion of laboratory-based surveillance to areas that lack the infrastructure to use standard methods.

**References**