

Greek measles epidemic strain, 2005–2006

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SUMMARY

The purpose of this work was the molecular study of the virus strain that caused the last measles outbreak in Greece. Twenty-four saliva specimens were obtained from selected patients serologically confirmed as measles cases between December 2005 and March 2006. Measles virus (MV) detection was performed by a nested RT–PCR. The 447-bp segment of the N gene of these MV strains was used for genotyping. The N gene sequences of the Greek MV strains were identical to each other, therefore a phylogenetic tree was constructed using one representative MV (ThesGRE/06). Our data show that the MV strain which caused the 2005–2006 outbreak in Greece belongs to genotype D6, and differs by 0·68 % from the New Jersey D6 strain and by 5·5 % from the MV vaccine strain Edmonston B (U03656) belonging to genotype A.

INTRODUCTION

Measles is a highly contagious disease and one of the leading causes of childhood morbidity and mortality. In 1998, the WHO European Region targeted elimination of measles by 2007 through vaccination programmes as vaccine coverage in excess of 95 % interrupts endemic transmission of measles [1, 2]. By this method, measles is successfully controlled in many parts of the world using live, attenuated vaccines, however, it still causes infections globally. In 2000, nearly 30 million infections were reported which resulted in more than 770 000 deaths [3].

In Greece, the MMR vaccine was introduced in the National Immunization Programme in 1989 and

the overall incidence of measles has since fallen. The vaccination scheme was reconsidered and changed twice since 1999, as two rubella outbreaks in 1993 and 1999 and a measles outbreak in 1995–1996 occurred. The current scheme consists of two doses of the vaccine, at 15 months and at 4–6 years of age [4].

The B' Laboratory of Microbiology in Aristotle University of Thessaloniki participated in a European programme for measles surveillance (ELSM; Enhanced Laboratory Surveillance of Measles) during July 2002 to July 2005 but no measles cases were reported in that period in Greece.

Since September 2005, a measles outbreak has been identified in Greece. It reached a peak in November and declined in March 2006. A total of 171 cases of measles were reported through the mandatory notification system of the Department of Surveillance and Intervention at the Hellenic Centre for Disease Control and Prevention (KEELPNO). Cases were

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reported from 14 of the 52 districts of Greece and 159 of the patients (93%) were from the northern part of the country. Most of the patients (119/171, 70%) belonged to gypsy and immigrant families and 60% were <9 years old. Of 110 patients with known vaccination status, 98 (89%) were unvaccinated for measles and 12 (11%) had had only one dose of the vaccine. A total of 103 patients (60%) were admitted to hospital, 27 (16%) had complications (mainly pneumonia and bronchiolitis), and all recovered [5].

The purpose of this work was the identification of the genotype of the measles virus (MV) strain that caused the outbreak, its position in a phylogenetic tree among other strains recommended by the WHO and its distance from the vaccine virus strain.

METHODS

Twenty-four saliva specimens were obtained from selected patients, serologically confirmed as measles cases (IgM positive) between December 2005 and March 2006. For each individual, data pertaining to age, sex, ethnicity, place of residence and history of MMR vaccination were obtained. Fifteen of the patients were children (mean age 4.4 years) and nine were young adults (mean age 30.8 years), 14 were male and 10 female, and 14 Greek and 10 of gypsy origin. The majority of them (75%) were unvaccinated, two had received only one dose of the vaccine and four had an unknown vaccination history.

Measles serology for the initial laboratory confirmation was performed by an ELISA assay detecting serum IgM antibodies (Genzyme Virotech GmbH, Rüsselsheim, Germany).

MV detection was achieved with nested RT-PCR. MV RNA was extracted directly from clinical samples using the viral RNA mini kit (Qiagen, Hilden, Germany), according to the manufacturer's protocol. Nested RT-PCR was performed on the N gene, by using the Superscript II One-step RT-PCR kit with Platinum Taq (Invitrogen Ltd, Paisley, UK) and primers as previously described [6].

RESULTS

MV was detected in 18 of the 24 clinical samples. The 447-bp segment of the N gene of these (18) MV strains was used for genotyping. Nucleotide sequences were aligned with respective measles sequences retrieved from GenBank (representative subtypes

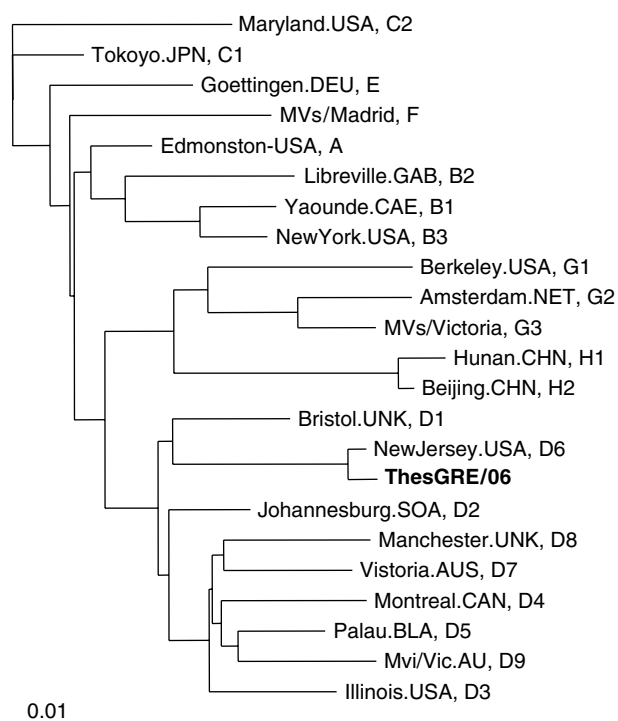


Fig. Phylogenetic relationships based on 447-nt region between the Greek measles strain (TheGRE06) and representative measles subtypes recommended by the WHO. The numbers indicate percentage bootstrap replicates (of 100) calculated using SWBOOT, DNADIST, FITCH and CONSENSE from the PHYLIP package; values below 60% are not shown. Horizontal distances are proportional to the nucleotide differences. The scale bar indicates 10% nucleotide sequence divergence. Vertical distances are for clarity only. The sequence obtained in this study is in bold.

recommended by the WHO) [7], and genetic distances were measured by the neighbour-joining method. The N gene sequences of the Greek MV strains were identical to each other, and a phylogenetic tree was constructed with PHYLIP (Phylogeny Inference Package by J. Felsenstein) using one representative MV Greek epidemic strain (TheGRE/06, accession no. DQ494347). The Greek strain clusters within the D6 genotype clade (Fig.). It differs by 0.68% from the New Jersey D6 strain and by 5.5% from the MV vaccine strain Edmonston B (U03656) belonging to genotype A.

DISCUSSION

Although MV is considered to be serologically monotypic, genetic differences have provided a basis for molecular epidemiological studies. There are currently eight recognized measles clades (A–H) and

23 genotypes [8]. Molecular epidemiology studies within the last 7 years have shown that the MV genotypes associated with outbreaks in several European countries were D6, C2 and D7 [9–13]. Only Santibanez *et al.* reported a case associated with D4 genotype in Europe, but because of a low degree of similarity with the D4 genotype viruses identified previously, the source of this MV remains unclear [10].

At present, the D7 genotype appears to be the most frequently detected in Western European countries; however, D6 genotype is still circulating in some European countries and the Russian Federation [9, 14]. It was the predominant genotype in an epidemic in Germany in 1996 and in Poland in 1998 [15]. It was also detected in the United Kingdom between 1992 and 1995 and in Spain in 1994 [14, 16]. Measles genotype D6 viruses were also imported to the United States from various European countries and Brazil on 13 occasions between 1997 and 2000; however, since 2000, only two D6 viruses have been detected in the United States [8]. During 2000–2001, wild-type measles viruses, classified as genotype D6, were also isolated in Turkey [17]. The same genetic variant (D6) was also circulating in several regions of Russia in 2003 and 2004 [18]. Since February 2005, an epidemic has been reported from Ukraine and the measles strain responsible belonged to genotype D6 and had 99.7% sequence homology in the 450 nt region of the N gene based on data received from two of the three European regional measles reference laboratories of the WHO [18].

Although, in some parts of Europe, measles is a disease close to elimination or has already been eliminated, in some areas is still endemic [2]. In Greece, measles incidence has been steadily declining during the past 25 years, due to an active vaccination programme in the country. According to several studies carried out in 2003–2005 in different parts of Greece, vaccination coverage of preschool children, schoolchildren and adolescents with one dose of MMR vaccine is >95% in the non-minority population and 80–90% in children of immigrant families. Vaccination with two doses covers about 60–80% of the general child population, but vaccination coverage in gypsy families has been found to be very low (2–12%) in studies of the period 2003–2005 despite the number of vaccination campaigns that took place in this group the previous years [19].

In conclusion, our data confirmed that the MV strain which caused the 2005–2006 outbreak in Greece belongs to genotype D6, it has a high degree

of homology with the New Jersey MV strain and its distance from Edmonston-wt strain is 5.5%. Additionally, the Greek measles outbreak may be linked to the current one in Ukraine as the appearance of a D6 virus in Greece might be due to a continued limited circulation of this genotype in central Europe.

Since serious respiratory and neurological complications are frequent, mostly in the young adults age group, efforts must be made and intensified to attain the 95% vaccine coverage in the area, not only in Greeks but in emigrants and gypsies as well, thereby the elimination of measles in Greece would be achieved.

DECLARATION OF INTEREST

None.

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