

## EDITORIAL

# Molecular epidemiology of measles in Liguria, Italy: a tool for the elimination of the infection

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## Key words

Measles • Molecular epidemiology • Measles elimination

The measles virus is an acute highly contagious viral agent that has a genome with negative polarity consisting of non-segmented single stranded RNA. It belongs to the Paramyxoviridae family in the order of Mononegavirales and only a single serotype is known [1]. Recently the World Health Organization (WHO) has described the genetic characteristic of wild-type measles virus into a total of eight clades and 23 genotypes [2]. It is one of the most infectious viruses known for humans and only considerably wide human populations are able to sustain circulation [1]. Despite the worldwide use of live vaccine, outbreaks of disease still occur in many countries including Italy [3-5]. In developing countries, as a result of the poor vaccination coverage, endemic circulation of measles is still ongoing causing a large number of deaths every year [6, 7]. Expanded Program of Immunization have increased the vaccine coverage in the world, which caused a dramatic decline in measles cases in many countries with substantial impact on reduction of measles morbidity and mortality, but elimination has proven to be difficult because of the recent development in transportation and the report of many imported measles cases from endemic areas [8, 9].

## Role of laboratory testing in WHO and National Plans for elimination of measles: contributions of molecular epidemiology for the control of virus infection

In March 2001, UNICEF and WHO released the document *Measles Mortality Reduction and Regional Elimination: Strategic Plan 2001-2005* with the main objective of reducing the number of global measles deaths by half by the end of 2005 compared to the total number of deaths in 1999 [10], of interrupting the endemic circulation of the virus by 2007 and of certifying measles elimination by 2010. This and the subsequent plan, *WHO European Region Strategic Plan 2005-2010: Elimination measles and rubella and preventing congenital rubella infection*, underlined the importance of laboratory testing for every suspected measles case. In 2002

was therefore initiated the European Region measles and rubella laboratory Network which is linked either to one of the three WHO European Region reference laboratories or to the global specialized laboratory located in the European Region. In Italy, the 2003-07 National Plan for elimination of measles and rubella recommends surveillance with laboratory testing in order: (i) to confirm diagnosis of acute measles infection especially when measles incidence is low and the clinical diagnosis become unreliable and (ii) to determine the virus genotype. Molecular lab activity implements the traditional epidemiology by helping both to determine the origin of the virus, the status of measles control and to verify the achievement of the *herd immunity* among surveyed population. Lab-based surveillance required implementation and standardization of diagnostic methods and reagents and a quality assessment programme, as widely recommended by WHO. When measles incidence was high, diagnosis were made without laboratory confirmation and, if it was done, it was traditionally based on methods such as immunofluorescence antibody assay for detection of viral antigen and haemagglutination inhibition, complement fixation or plaque-reduction neutralization tests for detection of measles antibody in serum [11]. Virus isolation can be performed on sensitive cell lines, such as B95a cells [12].

The introduction and the standardization of nucleic acid based assays, such as PCR, in the virological diagnostic in the 1990's provide an accurate and sensitive tool and represent the *gold standard* for virological surveillance and the patient management during an outbreak. Recently, real time RT-PCR assays for detection of measles virus genome were developed and proved to be useful in the routine laboratory surveillance because of their operating speed and their performance in terms of sensitivity, specificity and reproducibility [13, 14]. The implementation of RNA extraction procedure improved further upon the performance of molecular assays. The application of amplification techniques to sequence analysis allowed the diffusion of genome and phylogenetic characterization of viruses obtained from clinical samples.

As regards measles molecular characterization, WHO recommends the entire haemagglutinin-coding region (H) and 450 nucleotides encoding the COOH-terminal 150 aminoacids of the nucleoprotein (N) (which is one of the most variable parts of the measles genome), as the target for genetic characterization of RT-PCR products [2, 15, 16]. Several studies highlight the importance of investigating epidemic foci by sequence-based molecular methods that can reliably supply epidemiological information. Well-standardized surveillance networks together with virological studies may help to better document outbreaks of measles and might provide useful information on the molecular epidemiology of measles virus. Molecular characterization of measles viruses is an important component of measles surveillance and is more beneficial if it is possible to observe the change in virus genotypes over time in a particular region helping to document the interruption of transmission of the virus and providing an important method for assessing the effectiveness of vaccination programs. In particular, it is an important component of measles surveillance because allow (i) to establish epidemiological links with cases occurring simultaneously in other locations but also to exclude post-vaccination cases, (ii) to determine the localization of the index case also through retrospective testing of samples collected because of the high sensitivity of the PCR technique, (iii) to establish hypothesis as regards the imported origin of the strains of unknown origin.

An example of the application of molecular epidemiology is provided by the large measles outbreak occurred in the Americas during 1989-1991 with more than 55000 cases reported. In this case virologic and epidemiologic data collected during the following years indicated the interruption of transmission of the genotype D3 viruses associated with the outbreak that was subsequently maintained [17, 18].

### **Progress towards interruption of endemic measles transmission in Italy**

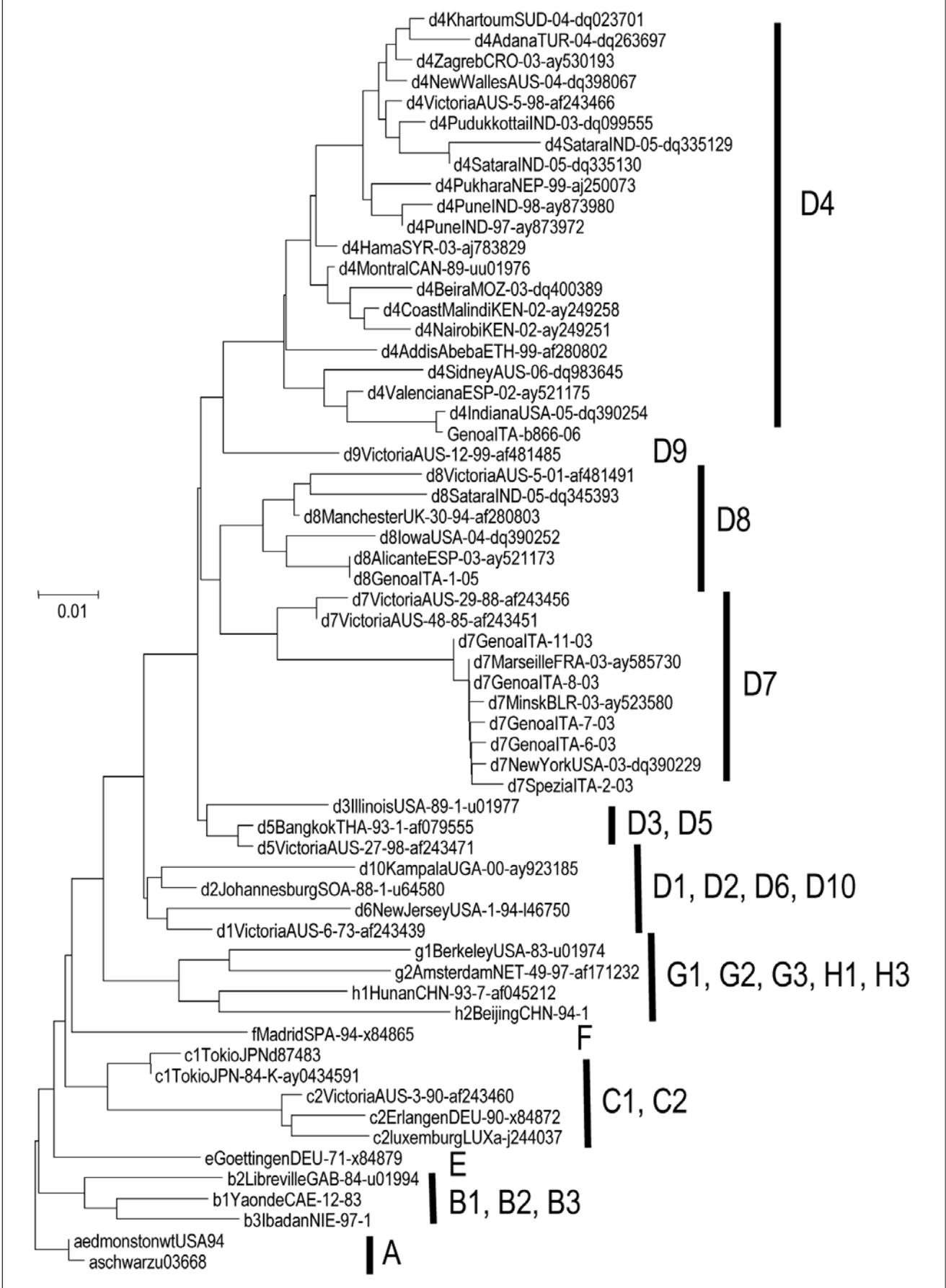
The elimination of measles requires high vaccine coverage in all age groups. In 1979 measles vaccination was introduced in Italy with monovalent vaccines and then, combined measles-mumps-rubella (MMR) vaccines were licensed [19] in 1990 and in the following years measles vaccination coverage increased, incidence declined and age shift was observed. However, measles epidemics continued to occur periodically: during 1995, 1997 and most recently in 2002 and 2003 [3, 4]. The Italian Measles National Elimination Plan, developed after 2002, recommends to reach a 95% first-dose coverage for achieving elimination and to conduct catch-up activities to reach high vaccination coverage [20]. The introduction of the second dose of measles vaccine seems necessary for achieving and maintaining elimination. Italy is one of the countries at high risk of measles epidemics because vaccine coverage remains lower than herd-immunity rate. Furthermore, a significant regional

heterogeneity in vaccine coverage and in epidemiological pattern is observed [21] and the distribution of cases in the country closely reflects the vaccination coverage distribution at provincial and regional level [22]. Since 2002 several outbreaks occurred in Italy and the most affected area was in southern regions. In 2002 an outbreak in Campania was reported [3]; in the following year there was a new rise in measles incidence mainly in Abruzzo, Puglia and Calabria [4], but an epidemic peak, with 187 cases, were reported also in Liguria; in 2006, outbreaks in Toscana [5] and, among gipsy [23, 24] communities, in Trentino Alto Adige, Lazio, Sardegna occurred. These outbreaks showed that measles continues to be a serious public health concern even in our country because of the sub-optimal and heterogeneous vaccine coverage.

### **Epidemiology of measles virus in Liguria: description of sporadic outbreaks**

An investigation conducted in Italy during 2003 [25] showed a measles vaccine coverage of 77%, with a significant increase respect the previous cross-sectional study performed in 1998 [26]. To implement the measles virological surveillance in Liguria, a network based on a case surveillance system with additional laboratory confirmation, including the 5 regional local health agency and Department of Health Sciences, was set up in 2003. During the spring 2003, 142 cases of measles were reported to the local health agency, with the highest incidence in the province of La Spezia. During the outbreak 16 respiratory samples of suspected measles cases were collected in Province of La Spezia and Genoa for laboratory confirmation. They were analysed by reverse transcriptase polymerase chain reaction (RT-PCR) and 8 of them were positive. Once genome was obtained, genetic characterization was performed, according to the international standards established by the global laboratory network [2, 15, 16]. As recommended by WHO, the entire Haemagglutinin-coding region (H) and 450 nucleotides encoding the COOH-terminal 150 aminoacids of the nucleoprotein (N) were the target for genetic characterization [2, 15, 16]. As shown in Figure 1, the phylogenetic analysis revealed that all samples shared the same cluster as genotype D7, showing 5% nucleotide difference in comparison with the reference strain. Genotype D7 circulated in Europe and was reported in several outbreaks in France, Germany and Belarus [27, 28]. During 2004 and 2005 measles case notifications in Liguria were sporadic, 6 and 4 respectively. In April 2005 one case was reported and laboratory confirmed. The genetic characterization showed that measles virus isolate belonged to genotype D8, that circulated in India and Nepal in the previous years and caused outbreaks in Spain; the foreign origin was hypothesized and the case was considered as imported. During the spring 2006, there have been 6 case notifications and one laboratory confirmed case, genetically related to genotype D4, that was original from Africa

Fig. 1. Phylogenetic tree of NP gene including WHO reference strains and virus isolated in Liguria.



and caused imported cases in the United States and Spain. The patient was a child of a gypsy community characterized by low level of vaccine coverage. Outbreaks in religious and nomadic communities with low level of vaccine coverage is one of emerging concerns during the present epidemiological phase characterized by low viral circulation and effort of infection control, as described by many authors [23, 24].

Therefore, epidemiological picture in Liguria is characterized by a sporadic circulation of the virus with only 12 cases in the last three years and a wide heteroge-

neity of isolated strains. In particular, the disappearance of genotype D7, that circulated in Europe during 2001-03, and the interruption of the circulation of this strain for a long period is due to the increasing vaccine coverage driven by Italian Measles National Elimination Plan, and the achievement of the herd immunity at regional level. Molecular characterization of the strains isolated in Liguria and in other European region showed a wide circulation of the virus introduced from Africa and Asia, due to a sub-optimal vaccine coverage in Europe.

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