

## ORIGINAL ARTICLE

# Epidemiological and molecular approaches for management of a measles outbreak in Liguria, Italy

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## Key words

Measles • Molecular surveillance • Syndrome surveillance

## Summary

*Since March 2010 a measles outbreak has been occurred in Genoa, Liguria, an administrative Region in Northern Italy. Epidemiological and molecular data on the outbreak, obtained from the passive mandatory notification system, the laboratory surveillance and an innovative syndrome surveillance system, were investigated. Overall 39 cases were reported in the urban area. Information about demography, vaccination status, hospitalization and geographic distribution of measles cases are available. 19 cases (48.7%) were laboratory-confirmed and were charac-*

*terized by sequence analysis: 18 strains belonged to genotype D8, so identifying a new measles variant within the Liguria population. Adopted control measures seem to have limited viral circulation. The outbreak allowed to test the efficacy of the 3 surveillance systems active in Liguria, highlighting their advantages and some important limitations. More efforts are needed to collect and integrate any epidemiological and virological available data in order to better describe the local measles transmission dynamics.*

## Introduction

In Italy a National Elimination Plan for Measles (MEP) has been implemented since 2003, aiming to reach a childhood vaccination coverage rate of 95%, to strengthen the disease surveillance and to definitively eliminate the indigenous transmission of measles [1].

Despite the coverage rate for first measles vaccine dose in children at two years of age has increased from 82.0% in 2003 to 90,1% in 2008 [2, 3] and the incidence of measles has decreased from 22.6/100.000 to < 1/100.000 in the same period [4], several measles outbreaks occurred in Italy during the last years [5-10].

In Liguria, an administrative Region in Northern Italy, the coverage rate for first measles vaccine dose in children at two years of age reached 86,8% in 2008 [11].

Since November 2007, a large outbreak spread in North-West Italy regions, following an imported case that developed symptoms 2 days after returning to Italy from the United Kingdom [5]. The number of reported cases by month in Italy increased from < 50 in September and October 2007 to > 100 in November, > 150 in December and > 400 in January and February 2008 [7]. The majority of the cases were reported in Piemonte, Lombardy, Tuscany, Emilia-Romagna and Liguria regions.

According to World Health Organization (WHO) measles program for European Region and the MEP, surveillance represents an essential component in the measles control and elimination strategy [1, 12].

In Italy, the measles surveillance is based on a passive reporting system: measles is a mandatory notifiable dis-

ease with exclusively clinical case definition and the physician who make diagnosis have to report the case to the Health Local Authority within 48 hours [1].

In 2000 an evaluation of the surveillance system has highlighted the limited completeness and comprehensiveness of notifications, showing underreporting of measles cases [13].

In the case of a measles outbreak, a laboratory confirmation of the first suspected cases have to be performed [1]: molecular epidemiology has been demonstrated to be a fundamental tool for epidemiologic investigation of measles, enabling the identification of source of infection and transmission pathways and the assessment of the efficacy of preventive intervention.

More recently, together with the routine surveillance systems, a pilot syndrome surveillance system has been implemented in Liguria and its performances have been tested, demonstrating high sensitivity and specificity in capturing measles cases and the capability to early detect an outbreak [14, 15].

Since March 2010 a measles outbreak has been occurred in the metropolitan area of Genoa, a city of 650.000 inhabitants, capital of Liguria. Below we report a description of the epidemiological and molecular data obtained from the three surveillance systems operating in Liguria.

## Materials and methods

Data were obtained from 3 principal sources, the routine mandatory notification system, a new syndrome surveil-

lance system, and molecular genotyping of virus by the regional reference laboratory.

### MANDATORY NOTIFICATION SYSTEM

In Italy, clinically suspected or laboratory-confirmed measles cases must be notified to the Local Health Units within 48 hours by means of a short form report [1]. Local Health Unit transmits the aggregated data to the Regional Epidemiological Observatory, to the Ministry of Health, to the Istituto Superiore di Sanità and to the Istituto Nazionale di Statistica [16]. Notified cases by Genoese Local Health Unit were obtained from the Data Computerized System for Communicable Diseases (SIMI), as of 15<sup>th</sup> May 2010 [17].

### SYNDROME SURVEILLANCE STUDY SITE AND POPULATION

In July 2007 a pilot Emergency Department (ED) syndrome surveillance system, based on data collected at “San Martino” University Hospital, began. “San Martino” Hospital is situated in Genoa and is the regional reference adult acute-care hospital. It was selected as a pilot site because it represents the largest hospital with the largest catchment area in Genoa, with an average of accesses to the emergency room of about 100.000 (range 96.000-120.000) per year and covering approximately 55% of all urban area ED visits. The main objectives of the surveillance system were to detect rapidly outbreaks of five syndromes, including measles-like illness, to activate quickly the epidemiological investigation and to facilitate a better public health response. Syndrome coding, data capture, transmission and processing, statistical analysis to assess indicators of disease activity and alert thresholds, and signal response were operatively described in Ansaldi et al. [9, 15]. Briefly, measles-like illness cases were defined as a patient with fever and

specific or non-specific diagnosis of maculo-papular rash compatible with an infectious disease, excluding allergic or inflammatory skin conditions such as contact or seborrheic dermatitis, rosacea and rash due to poison ivy, sun burn, and eczema [18, 19]. An indicator and a threshold value that can achieve optimum sensitivity and specificity in predicting relevant epidemiological events were established using the ROC curve approach. Syndrome surveillance data are presented plotting the normalized activity indicator for the epidemic threshold: eg, an activity indicator value of 2 corresponds to a number of ED visits equal to twice the epidemic threshold.

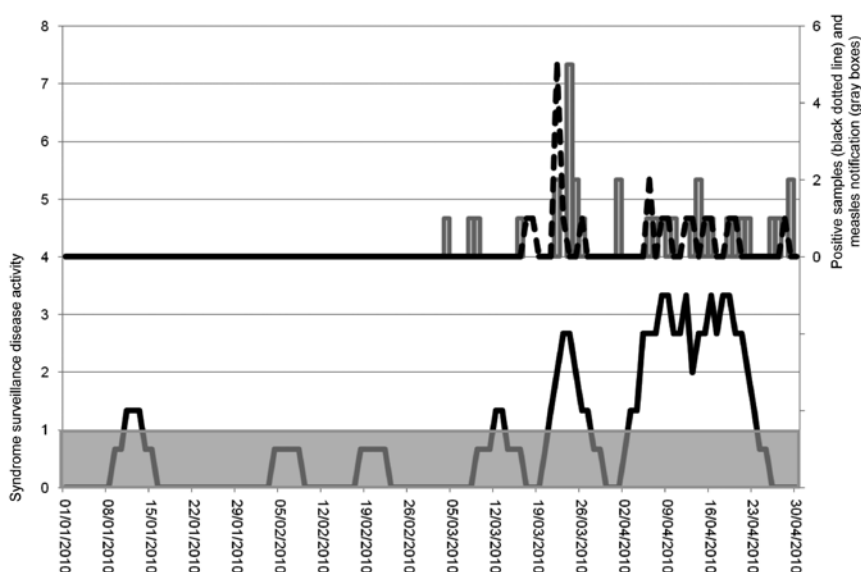
### LABORATORY SURVEILLANCE

The Italian National Plan for Measles Elimination, approved in November 2003, demanded laboratory confirmation for all suspected primary measles cases and viral molecular characterization when an outbreak occurs [1]. In Liguria the Regional Reference Laboratory for Measles and Rubella diagnosis, located at Department of Health Sciences, University of Genoa, has operated since 2003 and offers rapid detection by real-time and block RT-PCR, viral culture and genetic characterization by entire sequence analysis of Haemagglutinin-coding region (H) and 450 nucleotides encoding the COOH-terminal 150 aminoacids of the Nucleoprotein (N), in accordance with the international standards established by the global laboratory network [14, 20].

## Results and discussion

Syndrome surveillance indicator of measles-like illness activity, notified cases by Genoese Local Health Unit and measles-positive samples detected by the regional reference laboratory between 1<sup>st</sup> January and 30<sup>th</sup> April 2010 are reported in Figure 1.

**Fig. 1.** Syndrome surveillance indicator of measles-like illness activity, notified cases by Genoese Local Health Unit and measles-positive samples detected by the regional reference laboratory between 1<sup>st</sup> January and 30<sup>th</sup> April 2010.



From the beginning of March to the end of April 2010 a total of 39 cases were reported in the metropolitan area of Genoa: 33 cases were notified to the routine infection diseases surveillance system and 6 cases were only laboratory-confirmed and not notified to the authority. An intense circulation of measles was also registered by syndrome surveillance system: the activity indicator exceeded the epidemic cut-off for 30 out of 61 (49.2%) days.

During the previous 9 months, sporadic measles cases were notified or laboratory confirmed: from June to December 2009 only 5 notification, of which 4 laboratory confirmed, were reported by surveillance system. Syndrome surveillance indicator of measles-like illness activity occasionally exceeded the threshold value and breakthroughs never corresponded to measles virus detection.

Nineteen out of 39 cases (48.7%) were laboratory-confirmed and were characterized by sequence analysis: all but one Genoese strains were identical and belonged to genotype D8, while one case, confirmed on 19<sup>th</sup> March, belonged to genotype D4.

#### DESCRIPTION OF OUTBREAK

The first case, notified on 4<sup>th</sup> March 2010, was a 10-years old female child, living in the east administrative district of Genoa, admitted to the referral regional paediatric hospital (IRCCS G. Gaslini, Genoa) on 3<sup>rd</sup> March. The onset of symptoms was on 26<sup>th</sup> February.

On 2<sup>nd</sup> March the 13-years old brother complained first symptoms of measles and was not hospitalized; the case was notified on 8<sup>th</sup> March. In the first ten days of March further two cases occurred in two male children aged 7 and 9-years old and were notified on 9<sup>th</sup> and 16<sup>th</sup> March, respectively. The 9-years old case was admitted to the referral regional paediatric hospital. All these first cases were unvaccinated children. No clinical samples were available from these four cases for laboratory confirmation.

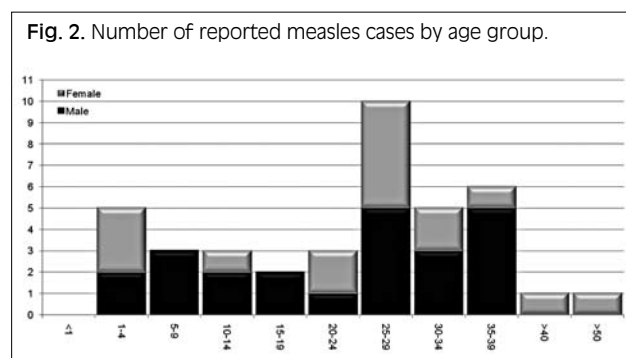
On 19<sup>th</sup> March a 1-year old female hospitalized case was laboratory confirmed but not notified to the authority.

The first two measles cases within not paediatric population were a 24-years old female and a 31-years old male admitted to the ED of "San Martino" Liguria Hospital on 22<sup>nd</sup> March. Both cases were laboratory confirmed during the following day and notified to the authority on 24<sup>th</sup> and 25<sup>th</sup> March, respectively.

Since 25<sup>th</sup> March further 32 measles cases were registered through at least one surveillance system.

The age was reported for all 39 cases. Overall, the median age of cases was 23.6 years (range: 1-53 years); 68.4% of cases were aged 15-39 years: more specifically, the most affected age groups was that of 25-29 years old with 26.3% of cases. Approximately the same percentage of cases were reported also for the 30-39 years old age group. The age distribution of cases is outlined in Figure 2. Male were more represented than female (56.4% vs 43.6%).

Adults aged 25-29 years had the highest incidence rate followed by children aged 1-4 years: 36.7/100.000 and 27.2/100.000, respectively.



Vaccination status was known for 76.9% of cases (30/39): 29/30 (96.7%) were unvaccinated against measles at the time of infection while the only one vaccinated case, was a two years old female that has received just one dose of measles-containing vaccine during 2009.

Data on hospitalization were available for all cases: 66.7% of subjects (26/39) required hospitalization.

Three of the first four paediatric cases lived in the east administrative districts of Genoa while the fourth case lived in the extreme north eastern peripheral district of the urban area. Similarly also the first adult cases came from the east administrative districts.

The outbreak subsequently spread to the central districts of Genoa, but no cases were reported from the west districts of Genoa. The geographical distribution of the cases occurred in the metropolitan area of Genoa is reported in Figure 3.

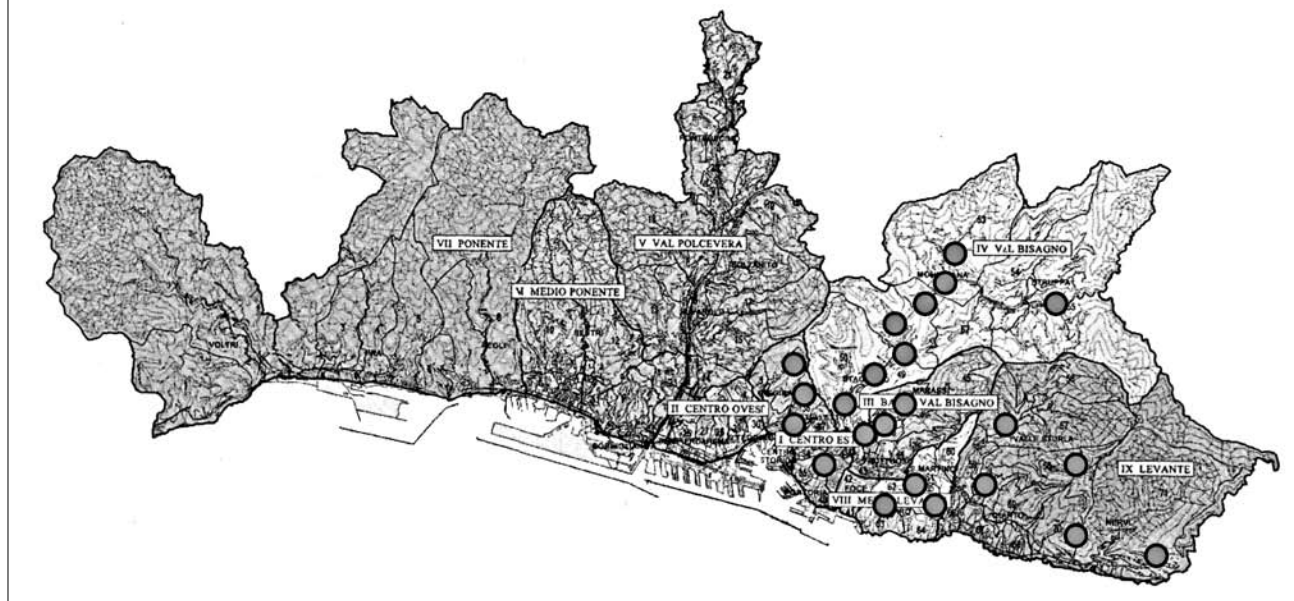
Local health authorities performed active tracing and vaccination of susceptible contacts in response to the described measles outbreak: measles containing vaccine was offered to all susceptible or incompletely vaccinated contacts of notified cases. Overall, 44 subjects were vaccinated in the metropolitan area of Genoa during March and April 2010.

General practitioners, family pediatricians and hospital physicians were also invited to report suspected measles cases to local health authorities as soon as possible.

#### SYNDROME SURVEILLANCE ACTIVITY

The activity indicator of maculo-papular fever rash broke through the epidemic threshold from 11<sup>th</sup> to 13<sup>th</sup> January 2010. Subsequently, the indicator was over the threshold on 12<sup>th</sup> and 13<sup>th</sup> March, from 21<sup>st</sup> to 27<sup>th</sup> March and from 3<sup>rd</sup> to 23<sup>rd</sup> April 2010, exceeding three times the epidemic threshold for 6 days (28.6%) during the last period. The January peak of activity did not correspond to measles circulation reported by other surveillance systems in Liguria. The subsequent threshold breakthroughs coincided with a sustained measles circulation in the metropolitan area of Genoa: in particular the peak registered by syndrome surveillance system on 12<sup>th</sup> and 13<sup>th</sup> March anticipated by ten days the first two laboratory confirmed measles cases within adult population. The paediatric cases reported at the beginning of March were correctly not captured by the syndrome surveillance system which is based on data collected from the ED of the regional reference adult acute-care hospital.

Fig. 3. Distribution of measles cases by metropolitan district of residence, city of Genoa, Liguria, Italy.



### MOLECULAR CHARACTERIZATION

Nineteen positive samples collected between 1<sup>st</sup> March and 30<sup>th</sup> April 2010 were characterized by sequence analysis. Phylogenetic analysis with MEGA software was performed on H and N gene nucleotide sequences and on the deduced amino acid sequences. The phylogenetic tree was built using the neighbor-joining method, including WHO reference strains and selected viruses isolated in different areas of the world. Figure 4 shows the D-genotype branch of COOH-terminal N gene tree. Nucleoprotein nucleotide sequence comparison and phylogenetic reconstruction indicating that 18 (94.7%) Genoese strains were identical and belonged to genotype D8 (cluster A in Figure 4). The Genoese strains belonging to cluster A showed 100% homology with measles viruses isolated during the same period in Piemonte, an Administrative region in Northern Italy adjacent to Genoese Local Health Unit region, and neighboring Local Health Units in Liguria. This strain was recently detected in India and during the last 7 years also in the United Kingdom and the U.S.A.

One Genoese strain belonging to genotype D4 was detected during the outbreak: it was identical to viruses isolated in the Liguria, Piemonte, Tuscany and Friuli Venezia-Giulia regions during a measles outbreak related to imported cases from the UK, that has been registered since November 2007 [5, 7].

Measles viruses detected in samples collected in Piemonte were also characterized. The phylogenetic analysis helped confirm the co-circulation of genotypes D4 and D8 already observed in Liguria: viruses in samples collected on 15<sup>th</sup> February, 10<sup>th</sup> March and 28<sup>th</sup> April 2010 belong to genotype D4, while the microorganisms found in the second half of March, 7<sup>th</sup> and 23<sup>rd</sup> April 2010 belong to genotype D8.

Phylogenetic analysis of H gene confirmed the above information (data not shown).

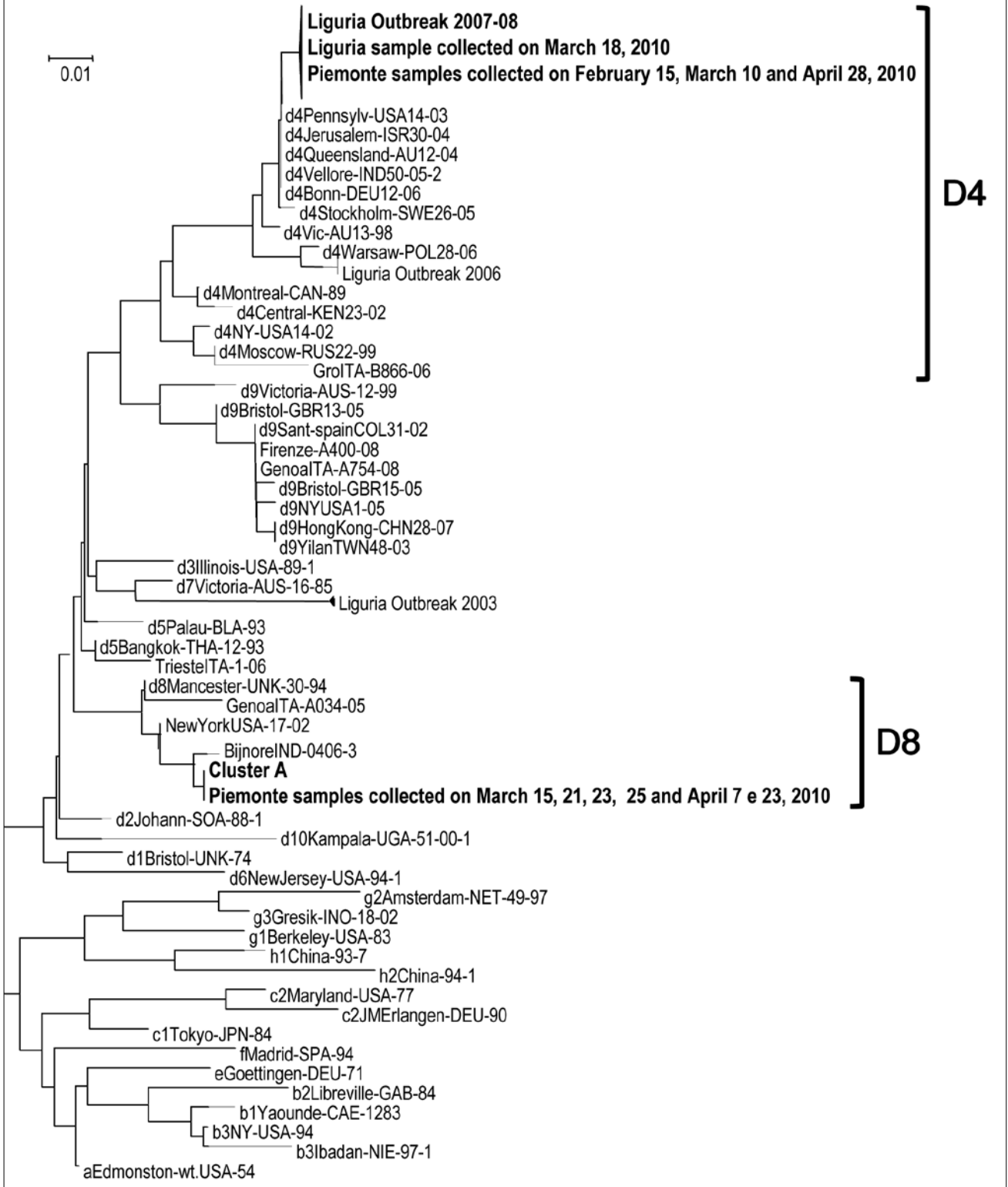
### Conclusions

The above described outbreak provides important information about the epidemiological picture of measles in Liguria. The relatively high coverage rates for first measles vaccine dose in children at two years of age (86.8%) [11] and the control strategies adopted by local health authorities, including contact investigation of cases, susceptible contact vaccination and implementation of isolation measures, seem to have limited spread of the outbreak, particularly in the city of Genoa, where a few eastern and central districts were affected (Figure 3).

The average age of cases was higher than that reported in other similar studies [6, 7, 10], with 26.3% of cases occurred in the 25-29 years old age group and more than 70% in subjects aged > 15 years. These data confirm the age shift in measles infection occurring in population with high percentage of vaccinated subjects in selected age groups (e.g. young children) and suboptimal coverage in the overall population. The circulation of measles virus in unvaccinated children further stresses the threat represented by pockets of susceptible populations and the urgent need to improve vaccination coverage with two doses of measles-containing vaccine in children, and to implement catch-up campaigns targeting adolescents and young adults: a recent paper reported that the coverage rate for the second dose at 15 years was 57.5% [11].

Also the hospitalization rate (66.7%) was higher than that registered by other authors [7, 10]. A possible reason explaining this data was the well-known underreporting of cases by general practitioners and paediatricians as compared to hospital physicians. The high hospitalization percentage highlights the need to improve effective infection control practices (e.g. active immunization of all susceptible healthcare workers, isolation measures, separate admission procedures for patients presenting measles-like syndrome) in order to prevent measles

**Fig. 4.** D-genotype branch of COOH-terminal N gene phylogenetic tree built using the neighbor-joining method, including World Health Organization reference strains and selected viruses isolated in different areas of the world.



transmission in healthcare settings. Data on notified case occupations appear to support this thesis: despite the small number of cases, 3 healthcare workers (9.1%) were affected during the described measles outbreak. The simultaneous presence of not notified laboratory-confirmed cases together with not laboratory-confirmed

notified cases underlines once more the lacks of the “traditional” surveillance system based on passive mandatory notification of measles cases. By contrast the innovative syndrome surveillance system confirms its high specificity and sensibility in anticipate the identification of measles outbreak and alert the public health institu-

tions, allowing the implementation of early and effective control strategies. The main limit of this system is the surveillance of only 55% ED accesses in the urban area of Genoa, in particular not covering the paediatric admission. For this reason, the syndrome surveillance system will be implemented with data collected from the ED of referral regional paediatric hospital (IRCCS G. Gaslini, Genoa).

The identification of a new measles variant within the Liguria population confirms the importance of molecular characterization of isolates. The laboratory surveillance allowed to characterize the new variant as D8 measles genotype, suggesting the introduction of this strain

during the second half of March 2010. These findings are confirmed by the characterization of recent isolates collected from Piemonte cases: all these data allow to hypothesize a broad circulation of this variant across the North western Italy.

This *on-field* experience illustrated both the limitations and advantages of traditional and innovative surveillance systems allowing to describe some important measles transmission dynamics. More efforts should be made to collect and integrate any epidemiological and virological available data in order to better describe source of infection, transmission patterns, geographical distribution and characteristics of the involved population.

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