ORIGINAL ARTICLE

# Measles surveillance activities in the Metropolitan Area of Milan during 2017-2018

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## Keywords

Measles surveillance • Measles laboratory confirmed cases • Measles epidemiological investigations • Indicators to monitor quality of
measles laboratory surveillance

## Summary

**Introduction**. *In Italy, the transmission of measles is still endemic, and 7,919 cases were reported to the National Surveillance System between January 2017 and December 2018. Aim of this study is to report the results of the measles surveillance activities in the Metropolitan City of Milan from March 2017 to December 2018, and to evaluate the surveillance performance WHO indicators.*

**Methods**. *The Local Health Units (LHUs) carried out case investigations and collected specimens to send to the EpiSoMI Lab (Subnational Reference Laboratory, SRL) of the University of Milan for cases/outbreaks confirmation and genotyping performed according to the WHO Guidelines.*

**Results**. *Overall, 610 suspected measles cases were reported by the surveillance system of the Metropolitan City of Milan. A total of 439 out of 540 cases with adequate specimens were laboratory-confirmed by molecular and/or serological assays. Two-hundred and thirty-six cases were notified as sporadic and 203 as related to 94 outbreaks. The most confirmed cases were aged 15–39 years, almost all not vaccinated. Overall, 282 cases were genotype D8 and 118 genotype B3.*

*The evaluation of a set of indicators to monitor the quality of surveillance activities demonstrated the proficiency of the EpiSoMI Lab.*

**Conclusions**. *A well-done investigation of cases and outbreaks by the surveillance local system, in a timely manner, in order to notify and investigate suspected cases and to laboratory confirm or discard cases is fundamental to reduce morbidity, to prevent further virus transmission and to achieve measles elimination.*

## Introduction

Measles is a highly contagious disease caused by measles virus (Mv). Humans are the only reservoir of this infection and an effective vaccine is available. These characteristics make this disease eradicable. The World Health Organization (WHO) planned to get the elimination of Mv, but the rapid succession of several measles outbreak in the European Region in the last years made this goal impossible to achieve in a short-term [1-3]. This is one of the leading immunization priorities of the European Region as outlined in the European Vaccine Action Plan 2015-2020 [4]. Elimination of measles will depend on achieving high coverage and closing immunity gaps and ensuring high-quality, case-based surveillance [5, 6]. In Italy, the transmission of measles is still endemic, and 7,919 cases were reported to the National Surveillance System between January 2017 and December 2018 [7, 8], including eight deaths. The median age of the cases was 26 years, but the highest incidence was recorded in children under one year of age, too young to be vaccinated. The main settings involved were hospital, family, school (included nursery and university), workplace and the community. In particular, numerous

nosocomial outbreaks have been reported [9, 10], highlighting both the problem of low vaccination coverage among health workers (among which 450 cases were reported) and the need to implement protocols for the prevention of measles transmission in healthcare [11]. In Italy, the Measles and Rubella Surveillance Network (Mo.Ro.Net), consisting of one National Reference Laboratory (NRL) and 14 Subnational Reference Laboratories (SRL) that meet rigorous standards to provide accurate results, was established in March 2017 [12]. Laboratories taking part in the network are required to participate in annual proficiency testing in selected techniques and are evaluated through the WHO accreditation program. The Laboratory of the Coordinated Research Center for the Epidemiology and Molecular Surveillance of Infections EpiSoMI (EpiSoMI Lab) of the University of Milan is one of the two SRL of the Lombardy Region (Northern Italy). The EpiSoMI Lab is a fully WHO-accredited laboratory and, from March 2017, set up a rapid and active surveillance for the complete characterization of the Mv in the Metropolitan City of Milan and surrounding areas.

A set of eight core indicators to monitor the quality of surveillance blend both field and laboratory activities [13]. Moreover, four of the eight indicators are di-

rectly related to the management and the performance of the laboratory. These four indicators are the reporting rate of discarded non-measles non-rubella cases, the laboratory confirmation, the viral detection, and the timeliness of reporting laboratory results [14]. These standard performance indicators should be monitored to identify weakness in the laboratory surveillance system so that corrective action can be taken [15].

Aim of this study is to report the results of the measles surveillance activities in the Metropolitan City of Milan and surrounding areas from 1 March 2017 to 31 December 2018. Furthermore, we want to evaluate the four indicators, directly related to the management and the performance of the laboratory, in order to demonstrate whether the routine surveillance laboratory activities provide accurate and timely data.

## Methods

### EPIDEMIOLOGICAL SURVEILLANCE DATA

In Lombardy Region, according to the National Surveillance Guidelines, all suspected measles cases must be promptly notified to the Local Health Units (LHUs). A suspected Mv case is defined as a subject with clinical evidence of "fever and rash" [6].

The LHUs carry out case investigations to determine source, risk factors and transmission settings, and conduct contact tracing to identify contacts, evaluate their immunity status, and vaccinate susceptible subjects. Moreover, LHUs collect specimens to send to the SRL for the case confirmation.

Notified cases are systematically reported to the Lombardy Regional database that provides, for each case, personal data, clinical details, all information collected during the epidemiological investigation as well as the SRL results including virus genotype.

Descriptive information on measles cases in this study were obtained from the Lombardy Regional database.

### LABORATORY SURVEILLANCE DATA

#### Specimen collection

Before collecting samples, informed consent was obtained by suspected Mv cases (or their legal tutors in case of minors). Collection of adequate specimens, therefore, may include collection of specimens to test for virus-specific immunoglobulin M (IgM) (by Enzyme ImmunoAssay, EIA, on serum, blood or Dried Blood Spot, DBS) and for measles RNA detection [by Real-time Reverse Transcription Polymerase Chain Reaction (RT-PCR) on urine and throat or nasopharyngeal swabs, Oral Fluid (OF)]. These biological samples must be collected during the acute phase of the disease, specifically between 4 and 10 days after the exanthema onset (between 4 and 28 days for DBS) for serological test, up to 14 days for OF or up to 21 days for urine for virus detection using molecular techniques, or in any case at the first contact with medical care [13-16].

#### Measles virus-specific IgM detection

Blood, serum and DBS samples received for IgM analysis were processed and tested as soon as possible after receipt in the laboratory. Serum samples were analyzed for serological testing using MV IgM capture Enzyme ImmunoAssay (EIA, Euroimmun AG, Luebeck, Germany), following the Manufacturing instructions.

#### Measles RNA detection

Total RNA was extracted from 1.5-15 ml of urine and/or 0.2-1 ml of OF, depending on the timing of specimen collection, using the NucliSENS® easyMAG™ automated platform (bioMérieux bv, Lyon, France) according to the off-board lysis protocol. Extracted RNA was analyzed for molecular testing using a One-Step Real-time PCR targeting the hemagglutinin (H) gene, as previously described [17].

#### Measles genotyping

The genotype of Mv strains was identified by sequencing the highly variable region of the nucleoprotein gene (N-450) [18]. RT-PCR products were purified with the NucleoSpin® Gel and PCR Clean-Up (Macherey-Nagel GmbH & Co. KG, Germany), and nucleotide sequences were obtained by automated DNA sequencing based on fluorescent dye terminator on genetic analyzer ABI PRISM 3100 Genetic Analyser (Applied Biosystem, Thermo Fisher, USA). N-450 Mv sequences detected during the seasons 2017-2018 were analyzed using the Basic Local Alignment Search Tool (BLAST, http://blast.ncbi.nlm.nih.gov/Blast.cgi) to identify similarities with previously reported strains and to define the belonging genotype. Virus genotypes were designated according to the official WHO nomenclature and sequences have been submitted to the WHO's MeaNS (Measles Nucleotide Surveillance) database [19, 20].

#### Laboratory confirmed case definition

A confirmed laboratory case was defined as a patient with serological and/or virological evidence of acute measles infection.

#### Indicators to monitor the quality of laboratory surveillance

The quality of laboratory surveillance and a sensitive system for detection and investigation of suspected cases of measles are evaluated by four performance indicators:

1. Reporting rate of discarded non-measles non-rubella cases: target: ≥ 2 cases per 100000 population per year. This indicator is calculated as the number of non-measles/non-rubella cases in a year divided by the average population in the studied area;

2. Laboratory confirmation: meaning the proportion of suspected cases with adequate specimens for detecting acute measles infection collected and tested in a proficient laboratory (target: ≥ 80%). This indicator is calculated as the proportion between the cases confirmed/discarded by each method of detection and the number of the suspected cases;

3. Viral detection: meaning the proportion of laboratory-confirmed chains of transmission (outbreaks) with samples adequate for detecting measles collected and tested in an accredited laboratory (target: ≥ 80%). This indicator reflects the fundamental contribution of the molecular characterization of the Mvs and is calculated as the percentage of all chains of transmission, identified during a calendar year, that have been successfully characterized by genetic analysis;

4. Timeliness of reporting laboratory results: meaning the proportion of results reported by the laboratory within 4 days of specimen receipt (target: ≥80%).

### STATISTICAL ANALYSIS

The comparison of two proportions was analyzed using the Chi square test. Two-sided p values < 0.05 were considered statistically significant. All analyses were conducted using the OpenEpi software [21].

## Results

### LABORATORY SURVEILLANCE DATA

From March 2017 to December 2018, 610 suspected measles cases were reported by the surveillance system of the Metropolitan City of Milan and surrounding areas. For 540 (88.5%, 540/610) measles suspected cases were collected adequate specimens for serological and/or virological confirmation by the SRL. Of the 540 suspected cases, 80 (14.8%) had specimens only to be tested by molecular tests, whereas 6 (1.1%) only for serological evaluation. A total of 439 (439/540, 81.3%) reported cases were laboratory-confirmed by molecular and/or serological assays, in accordance with the WHO guidelines [12] (Fig. 1). The 59.2% (260/439) of confirmed cases tested positive by both serological and molecular assays; the 39.9% (162/439) was confirmed only by molecular tests, and the 3.9% (17/439) exclusively for anti-Measles IgM (Tab. I).

In Figure 2 is described the number of confirmed and discarded cases by age groups and year of surveillance. During the considered period, the proportion of discarded measles cases was 45.2% (38/84) for the age groups 0-4 years, 17.9% (7/39) for the age groups 5-14 years, 11.03% (32/290), 10.7% (12/112) and 75.0% (9/12) for the age groups 15-39, 40-64, and ≥ 65 years respectively. A statistical significance was found in the rate of discarded non-measles cases of age groups 0-4 and ≥ 65 years (both p < 0.005).

Overall, 58.8% (258/439) of the confirmed cases (median age: 28 years; range: 1 day-7 years) were aged 15-39 years, 22.8% (100/439) belonged to the 40-64 age group, 10.5% (46/439) were aged 0-4 years, and 3.4% were ≤ 1 year old. The 93.3% of confirmed cases were not vaccinated. During the considered period, the epidemiologic investigation identified 94 different outbreaks/chains of transmission: 77 during 2017 and 17 during 2018 epidemic. The 53.76% of the confirmed cases was notified as sporadic.

Four-hundred and thirty-four out of 439 confirmed cases had adequate specimens for viral detection and 402 out of 434 (92.6%, 402/434) cases were genotyped. In 2 subjects, vaccinated as susceptible during the epidemic period, was identified genotype A vaccine strain (0.5%). These subjects were excluded, since did not meet the case definition (genotype identification is required to distinguish wild type from vaccine strain if vaccinated within 21 days of rash onset). Two different genotypes were identified, D8 and B3. Genotypes D8 and B3 have co-circulated during the whole period (Fig. 3).



Fig. 1. Measles cases investigated during the period of surveillance activity to the enhanced measles surveillance system (data from the official data base of Lombardy Region) and cases confirmed by the SRL EpiSoMI, March2017-December 2018.
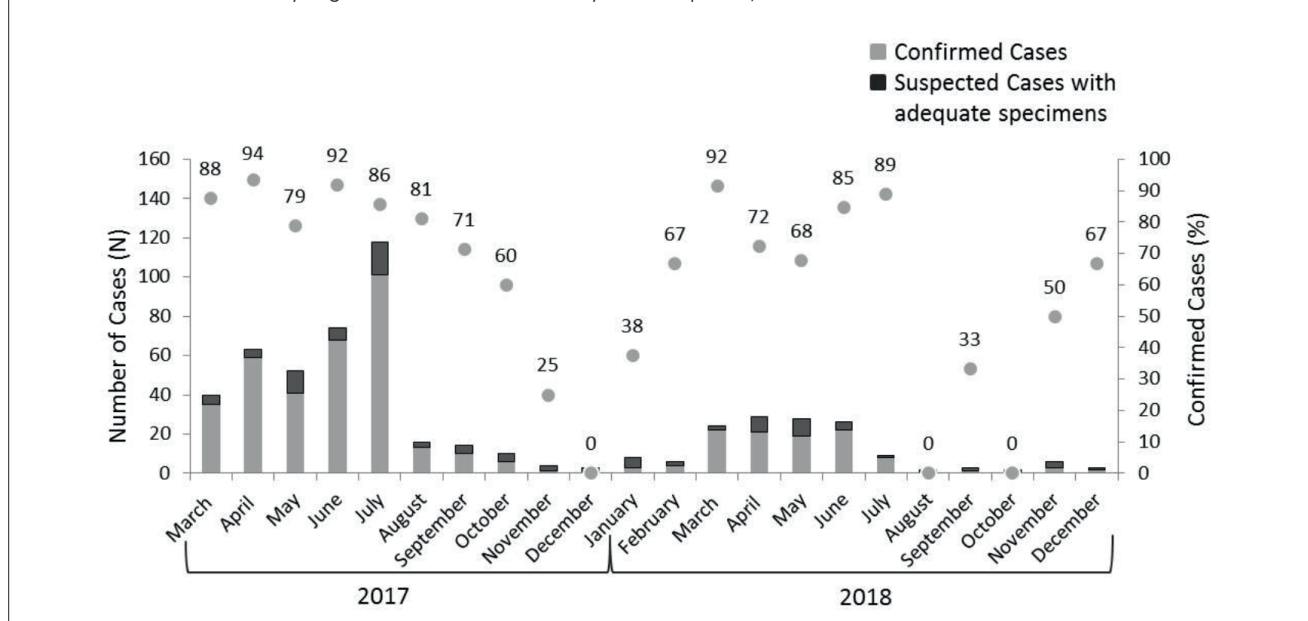
Tab. I. Reported suspected cases, suspected measles cases with adequate specimens and cases confirmation distribution during the study period (2017 and 2018).

| Parameters | | 2017* | 2018 | Study period, N |
|---|---|---|---|---|
| Reported suspected measles cases, N (%) | | 440 (72.1) | 170 (27.9) | 610 |
| Suspected measles cases with adequate specimens to be tested, N (%) | | 394 (73.0) | 146 (27.0) | 540 |
| Number of different specimen type investigated, n (%) | Serum/Blood/DBS | 372 (68.9) | 168 (31.1) | 540 |
| | Urine | 413 (69.3) | 183 (30.7) | 596 |
| | Oral fluid | 405 (69.8) | 175 (30.2) | 580 |
| Measles cases confirmed by lab, N (%) | | 335 (76.3) | 104 (23.7) | 439 |
| Assays used to confirm measles case (%) | Serological | 11 (64.7) | 6 (35.3) | 17 |
| | Virological | 125 (77.2) | 37 (22.8) | 162 |
| | Serological and virological | 199 (76.5) | 61 (23.5) | 260 |

* in 2017 the parameters evaluation started on March.

Fig. 2. Distribution by age groups for confirmed/discarded cases during surveillance activity of SRL EpiSoMI, March 2017- December 2018.
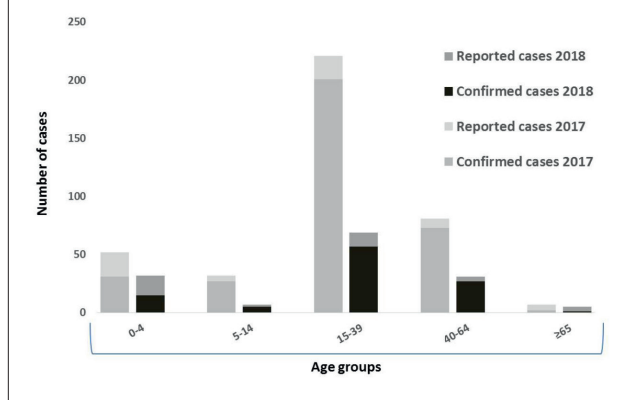


The most common genotype was D8 (70.5%, 282/400 cases), while B3 genotype was identified in the 29.5% (118/400) of cases. D8 genotype was mainly observed during 2017 epidemic (92.6%, 261/282), whereas during 2018 the most circulating genotype was the B3 (76.4%, 68/89).

Overall, D8 genotype was identified in 277 (98.2%, 277/282) autochthonous and 4 imported cases. In particular, 152 (53.9%, 152/282) were notified as sporadic cases, while 130 (46.0%, 130/282) were involved in 68 outbreaks. B3 genotype was identified as imported in 13 cases out of 118 (11.0%, 13/118), as imported-related in 1 case and as autochthonous in 105 (89.0%, 105/118) cases. Otherwise, epidemiological investigation notified 61 (51.7%, 61/118) cases as sporadic and 57 (48.3%, 57/118) cases as related to 24 outbreaks.

### INDICATORS TO MONITOR QUALITY OF LABORATORY SURVEILLANCE

The four indicators to monitor the quality of laboratory surveillance are calculated by period (March 2017-December 2017 and January 2018-December 2018) and the results are shown in Table II.

The number of suspected cases that met the clinical case definition but were not laboratory confirmed was 60 during 2017 and 39 during 2018. Considering a mean population of 4000000 inhabitants included in the Metropolitan City of Milan and surrounding areas, the reporting rate of discarded cases was about 1.5 per 100000 popu-

Fig. 3. Genotypes circulation in the Metropolitan Area of Milan, from March 2017 to December 2018.
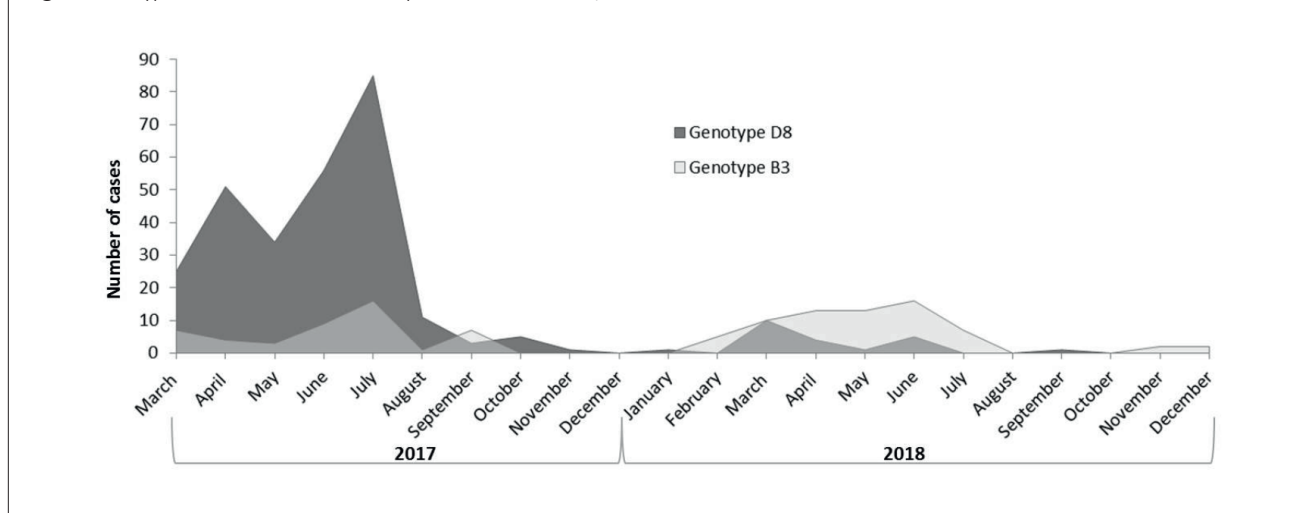
lation per year in 2017 and about 1 per 100000 population per year in 2018.

In the considered period, more than the 80% of all cases that met the clinical definition had the collection of adequate specimens and were laboratory confirmed/discarded. Viral detection and genetic characterization of measles virus responsible of a chain of transmission was about 99% (only two chains of transmission were not genetically characterized). The 89.6% (484/540) of laboratory confirmed cases were reported to LHU by the laboratory within 4 days of specimen receipt.

## Discussion and conclusions

Monitoring progress toward measles elimination requires high-quality case-based surveillance that is able to, in a timely manner, detect, notify and investigate suspected measles cases and outbreaks, correctly classify them as confirmed or discarded, and prevent further virus transmission [5, 22]. A key role of the high-quality case-based surveillance is represented by the laboratory activities.

In order to increase the performance level requested by the WHO, including timeliness and completeness of data, in March 2017 a sub-national network of accredited laboratories for measles and rubella surveillance (Mo.Ro.Net) coordinated by the NRL was formalized [12, 23].

As SRL, EpiSoMI Lab undergoes regular and thorough processes for monitoring the accuracy and performance of its procedures and the operators who performing them (WHO accreditation). The EpiSoMI Lab must maintain that status for the forthcoming calendar year, thus the accreditation assessment is based on the laboratory's performance during the preceding 12 months. EpiSoMI Lab achieved full accreditation for both investigated years, 2017 and 2018.

In the Lombardy Region, from September 2013 to May 2014 were collected biological samples from 80 suspected cases and the 57.5% were laboratory confirmed. In that period, in Lombardy were noticed 880 suspected cases [24], while in 2016, 165 Mv cases were notified and 114 (69.1%) were confirmed (unpublished data). From March 2017 (date of Mo.Ro.Net establishing) to December 2018, a total of 959 Mv cases, including

confirmed, possible and probable cases, were reported in Lombardy Region [25]. In particular, 610 out of 959 Mv cases was related to a large measles outbreak in the Metropolitan City of Milan and surrounding areas, a highly populated area with about 4 million inhabitants. In this area, 439 measles cases (81.3% of cases investigated) were laboratory-confirmed. This highlighted how a well-organized accredited laboratory improves the quality of the surveillance system. The laboratory needs adequate clinical samples to be timely and accurate in reporting confirmation/discard for clinically suspected cases. The detection of virus-specific IgM is the standard method for case confirmation. However, the use of RT-PCR for direct detection of measles specific RNA is fundamental to complement IgM antibody detection. Using PCR method has become necessary to perform a rapid case/outbreak investigation and to adopt the "fever and rash" case definition by collecting specimens at the first encounter with the healthcare system, and performing molecular testing for virus detection in a "fast and aggressive" way [13, 26, 27]. Remarkably, with this strategy, the EpiSoMi Lab, during 2017-2018 period, have confirmed by molecular methods a relevant proportion (13.2%) of seronegative cases (< 4 days from rash). These results prove the value of molecular analysis as tool to identify Mv cases otherwise unrecognized.

Overall, the 439 confirmed cases were classified by epidemiological investigations: 236 as sporadic cases and 203 as related to 94 outbreaks. Four hundred cases were genotyped: 282 were genotype D8 and 118 were genotype B3. These two genotypes co-circulated in Europe during the study period [28].

A set of indicators was identified by the WHO [14] to monitor the quality of surveillance activities, including the reporting rate of discarded non-measles non-rubella cases (≥ 2 cases per 100000 population per year). The 88.57% of suspected Mv cases were laboratory confirmed with a reporting rate of discarded non-measles cases between 1-1.5 per 100000 population in the two years, whereas at national level the reported rates were of 0.67 per 100000 population per year in 2017 and of 0.37 per 100000 population per year in 2018. The rate of discarded Mv cases were significantly higher in 0-4 years and ≥ 65 years age groups. These results could be explained by a higher attention and sensitivity for pediatric population (at higher

Tab. II. Indicators of the quality of laboratory surveillance.

| Laboratory indicators | Results | | Target |
|---|---|---|---|
| | March 2017 - December 2017 | January 2018 - December 2018 | |
| 1. Reporting rate of discarded non-measles non-rubella cases | 1.5 cases per 100000 population per year | 1 case per 100000 population per year | ≥ 2 cases per 100000 population per year |
| 2. Laboratory confirmation | 89.5% | 85.9% | ≥ 80% |
| 3. Viral detection | 100% | 97.4% | ≥ 80% |
| 4. Timeliness of reporting laboratory results | 88.9% | 92.4% | ≥ 80% |

risk of clinical complications, also neurological), and by a very low measles incidence in the elderly.

Another critical indicator of surveillance performance is the viral detection and genotyping (≥ 80% of laboratory-confirmed chains of transmission) [29]. The mean rate of genomic characterization performed during the observed period is 98.7%, much more than the national average (61.5% for 2017) [11]. The genetic characterization has enabled us to identify or confirmed epidemiological links.

The evaluation of these indicators is intended to demonstrate the proficiency of the accredited laboratories and whether adequate surveillance laboratory activities are implemented and documented for verification purposes. These data demonstrate that the EpiSoMI Lab has supported Mv cases ascertainment in the Lombardy Region in these two years of activity in Mo.Ro.Net in a proficient way, confirming outbreaks/cases and determining Mv circulating genotypes.

Achieving indicator targets provides assurance that public health authorities can detect, locate and describe potential Mv transmission in a timely manner [26].

In conclusion, a well-done investigation of cases and outbreaks by the surveillance local system, in order to notify and investigate suspected cases and promptly laboratory confirm or discard cases, is fundamental to reduce morbidity, to prevent further virus transmission and to achieve measles elimination.

## Acknowledgements

## Conflict of interest statement

None declared.

## Authors' contributions

SB Study design, project and protocol development, coordinated and contributed to the laboratory testing, data analysis and manuscript writing.

MF coordinated the activity of the Health Protection Agency of Metropolitan Area of Milan (Milan, Italy), and critically revised the manuscript.

SS and AL coordinated the epidemiological surveillance activities, contributing substantially to the acquisition of the epidemiological data, and contributed to the writing of the manuscript.

DCo and ERF protocol development, laboratory testing and critically revised the manuscript

GC and MGo laboratory testing and critically revised the manuscript.

MGr and DCe secured study funding, acquisition and analysis of the epidemiological data.

FA contributed to the development of the local surveillance activities and critically revised the manuscript.

ET contributed to the conception and design of the study and critically revised the manuscript.

AA coordinated and supervised the research, designed the study and write the manuscript.

All authors revised the manuscript and contributed to improving the paper. All authors read and approved the final manuscript.

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