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SHORT COMMUNICATION

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Differential diagnosis on measles and rubella discarded cases highlights a sharp increase in parvovirus B19 infections in Milan, Northern Italy, in the first months of 2024

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Abstract

In line with European trends, since 2023 Lombardy (Northern Italy) is experiencing a resurgence of measles and an increased number of reported cases of fever and rash. Measles discarded cases observed in our region within the context of measles and rubella surveillance from the first few months of 2024 ($N = 30$) were investigated for parvovirus B19 (B19V) and other rash-associated viruses. Thirteen cases tested positive for B19V DNA, representing a significant increase from previous years (on average 3 cases per year, $p < 0.001$) and ~40% of all B19V DNA-positive patients we detected since 2017. In 2024, B19V DNA-positive subjects spanned all ages, and the virus was predominant among adolescents and adults (84.6%). Two B19V infected patients were hospitalised, and likely cross-reacting anti-measles virus IgM were found in both. Our data align with the recent reports from the ECDC and various European countries, which are experiencing a surge in B19V infections, and underline the importance of comprehensive measles and rubella surveillance systems that can adapt to changing epidemiological trends.

KEYWORDS

differential diagnosis, fever and rash, measles surveillance, parvovirus B19

1 | INTRODUCTION

Lombardy, a Region in Northern Italy, has recently experienced a marked increase in the number of measles suspected and confirmed cases compared to last year.[1] This is in line with the alarming resurgence of measles documented by the World Health Organisation (WHO) European Region[2] and the European Centre for Disease Prevention and Control (ECDC),[3] who report already for the first few months of 2024 almost as many measles cases as those reported in the whole 2023.[4]

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Likewise, as a subnational reference laboratory for measles and rubella surveillance in the Metropolitan City of Milan and surrounding areas (Lombardy), between January and May 2024, we investigated 54 cases of suspected measles, compared to 19 cases analysed in the whole 2023. These were all patients who presented with fever and rash ("fever and rash strategy").[5] Twenty-four of these cases (44.4%) were molecularly and/or serologically confirmed as measles.[6]

In this epidemiological scenario, as well as in light of the WHO measles elimination goal, it is of utmost importance to perform differential diagnosis of the cases suspected to be but not confirmed as either measles or rubella (discarded cases) to identify other pathogens that may be responsible for this clinical profile. Particularly, measles and parvovirus B19 infections may sometimes present clinically quite similar (maculopapular rash), which calls for an accurate laboratory diagnosis, particularly when these viruses co-circulate together.[7] For this reason, the 30 discarded cases from the first 5 months of 2024 were further investigated for other viral pathogens associated with morbilliform rash, including human herpesvirus 6 (HHV-6) and 7 (HHV-7), parvovirus B19 (B19V), enterovirus (EV), Epstein–Barr virus (EBV), human adenovirus (HAdV), and cytomegalovirus (HCMV). In this cross-sectional investigation, we describe an increased detection of B19V during the first few months of 2024, compared to previous years, in agreement with other European reports.[8,9]

2 | MATERIAL AND METHODS

The area under surveillance included the metropolitan area of Milan and its surroundings, a densely populated area of about 4 million inhabitants, as well as other provinces in Lombardy, i.e., Brescia, Varese, Como, and Monza-Brianza.

Measles and rubella suspected cases were defined as discarded according to the WHO definition: fever and rash cases that tested negative to both molecular assays aimed to detect measles virus (MeV) and rubella virus (RuV) RNA in oropharyngeal swabs, blood, or 10–50 mL of urine, and to specific immunoglobulins type M (IgM), investigated in sera collected during the acute phase of infection (1–10 days after the rash onset).[6] Measles and rubella molecular and serological confirmation tests were performed according to the WHO laboratory manual.[6] Enzyme-linked immunosorbent assays (ELISA) were performed using a commercial kit (Euroimmun, Germany).

The oropharyngeal swabs (Copan, Italy) of the discarded cases were tested for B19V and other rash-associated viruses (HHV-6, HHV-7, EV, EBV, HAdV, and HCMV). Nucleic acids were isolated using NucliSENS® MiniMAG™ system (bioMérieux, France) and tested through a commercial TaqMan-Based Multiplex Real-Time PCR kit (Siemens Healthineers, Germany) as previously reported.[5]

The analysis also draws upon data collected from March 2017 and December 2022 reported by Fappani et al.[5]

Median age as well as the interquartile range (IQR) were calculated for the patients and statistical significance was assessed using the Monte Carlo or Fisher's exact test (2 tails).

3 | RESULTS

In 2024 (January to May), 13 out of 30 measles/rubella discarded cases (43.3%) tested positive for B19V DNA. These cases represented 39.4% of all B19V DNA-positive subjects ($N = 33$) that we detected among the 329 discarded cases investigated in the past 8 years, since the beginning of surveillance activities (full results for March 2017–December 2022 are available in Fappani et al.[5]) (Table 1). In the first 5 months of 2024, we experienced a slight increase in the number of reported fever and rash cases, which had previously dropped dramatically in 2020, after the onset of the COVID-19 pandemic. Furthermore, a high proportion of discarded cases tested positive for B19V DNA (Figure 1). The number of B19V DNA-positive patients remained low (on average ~3 cases/year) until December 2023 (Figure 1) and a significant difference in positivity rates between the various years was observed ($p < 0.001$ with Monte Carlo exact test). No seasonal trend was observed and in six patients (46.2%), B19V was co-detected with HHV6 ($N = 1$), HHV7 ($N = 3$), HHV6 and HHV7 ($N = 1$), or HSV-1 and EBV ($N = 1$).

B19V DNA was recorded among both children and adults, with an overall median age of infected patients of 33 years (range 0–66 years, IQR 5–43 years) (Table 1).

Of note, the proportion of adolescents and adults (15–64 years) among B19V DNA-positive patients observed in the first 5 months of 2024 was higher, although not significantly, than during the previous years (11/13, 84.6% vs. 10/20, 50%, $p = 0.07$ with Fisher's exact test).

None but two B19V DNA-positive subjects from 2024 were hospitalised. The patients were 40 and 47 years old, and one of them experienced neurological symptoms.

Serum samples from the two B19V DNA-positive hospitalised patients from 2024 tested positive for anti-measles virus (MeV) IgM antibodies using the chemiluminescence immunoassay technology performed at the admitting hospitals (data not available). However, both samples were only weakly positive when we performed anti-MeV IgM ELISA (obtained values were coinciding with or slightly above the cut-off of 1.1: 1.1 and 1.2), and MeV RNA was not detectable in oropharyngeal swabs or urine samples collected during the acute phase of infection (4 and 5 days after the rash onset) from these patients. These discordant results could be explained by an antigenic cross-reaction. Serum samples from the other 11 B19V DNA-positive patients all tested negative for anti-MeV reacting antibodies.

4 | DISCUSSION

In April 2024, the ECDC alerted on the increase of B19V infections in several EU/EEA Member States.[8] Since then, 14 EU/EEA Member States (i.e., Czechia, Finland, France, Germany, Hungary, Ireland, Italy,

TABLE 1 Number of fever and rash and measles/rubella discarded cases and parvovirus B19 (B19V) DNA-positive patients identified during each surveillance year and stratified by age.

	Fever and rash cases	Discarded cases[a]	B19V-positives cases (%)					
			Total (%[b])	0–4 years (%)[c]	5–14 years (%)[c]	15–39 years (%)[c]	40–64 years (%)[c]	>64 years (%)[c]
2017	418	78	3 (3.8)	1 (33.3)	0	1 (33.3)	0	1 (33.3)
2018	181	74	4 (5.4)	1 (25.0)	1 (25.0)	1 (25.0)	1 (25.0)	0
2019	334	104	6 (5.8)	2 (33.3)	1 (16.7)	1 (16.7)	2 (33.3)	0
2020	17	11	1 (9.1)	0	0	0	1 (100.0)	0
2021	19	18	3 (16.7)	2 (66.7)	0	0	1 (33.3)	0
2022	9	4	0	0	0	0	0	0
2023	19	10	3 (30.0)	1 (33.3)	0	2 (66.7)	0	0
2024[d]	54	30	13 (43.3)	0	2 (15.4)	7 (54.8)	4 (31.8)	0
Total	1032	329	33 (10.0)	7 (21.2)	4 (12.1)	12 (36.4)	9 (27.3)	1 (3.0)

[a]Number of measles and rubella discarded cases for which samples were still available.
[b]Percentage of discarded cases that tested positive for B19V DNA.
[c]Percentage of B19V DNA-positive patients in each age group.
[d]January-May 2024.

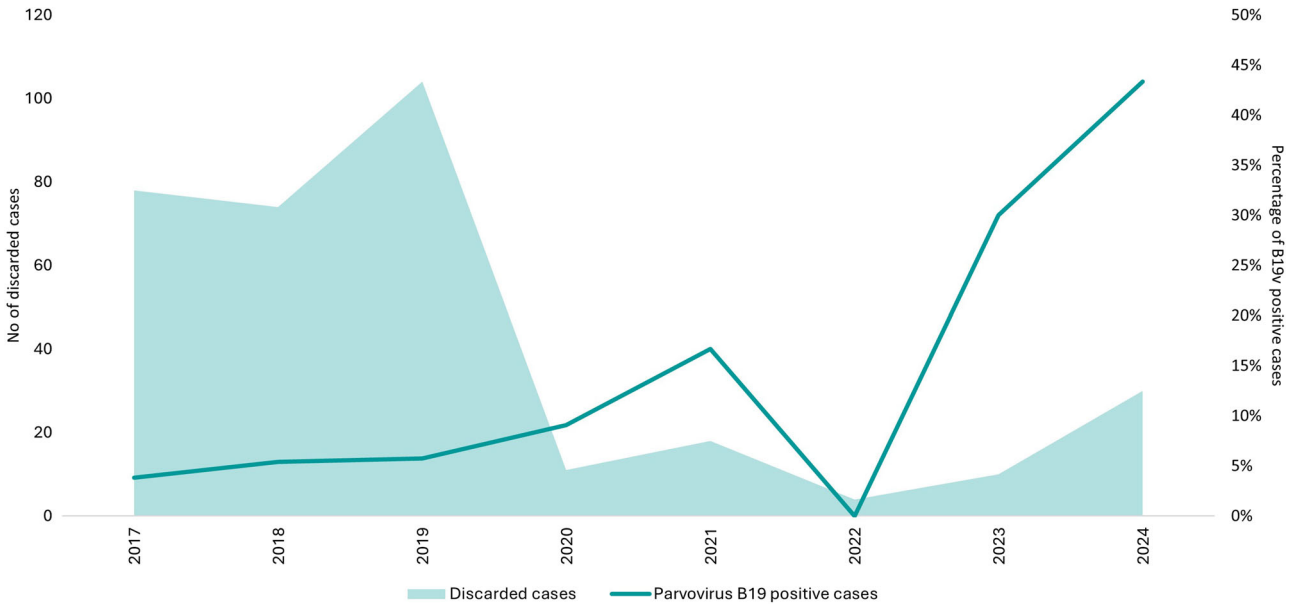

FIGURE 1 Number of measles and rubella discarded cases and proportion of discarded cases that tested positive for parvovirus B19 (B19V) DNA between March 2017 and May 2024.

Latvia, Lithuania, Luxembourg, the Netherlands, Norway, Slovakia, and Spain) have reported increased detections of B19V through various surveillance systems.[9] Among them, 10 countries (Finland, Hungary, Luxembourg, Lithuania, the Netherlands, Czechia, Denmark, France, Germany, and Slovakia) reported an increase in reactive tests for B19V in blood donors or in donations of plasma during the first months of 2024 compared to the same period in 2023.[9] In particular, Guillet and colleagues reported a 10-fold increase in B19V detection in blood donations during the period December 2023–March 2024 *versus* the pre-pandemic period.[10]

While infected patients belonged to several age groups, young children were the most affected.[7,8]

Of note, the Greek Public Health Organization (EODY) warned of several B19V cases among children in Thessaloniki, including a child who passed away after developing acute myocarditis due to B19V (ΚΠ 8456/2024 01/05/2024). Indeed, as seroprevalence of

anti-B19V antibodies due to prior infections increases with age (5–10% in young children, 50% in young adults, and more than 90% in the elderly), the probability of postexposure infection is higher in younger individuals.[9] However, our results are more in line with later reports from Norway that highlighted an increase in B19V-positive results in the adult population (30–59 years).[8,9] The increase in positive B19V infections among adults constitutes a worrisome trend considering the important consequences of B19V infection on the foetus when contracted during pregnancy.[11] Although none of the B19V DNA-positive patients we detected were pregnant women, our results warrant us to remain vigilant and increase B19V testing during pregnancy. Nonetheless, we need to point out that only samples from patients with measles/rubella-like symptoms were available for our study and only discarded cases were tested. Therefore, our detections likely represent a small proportion of the total number of B19V infections occurring in the territory under surveillance, particularly considering that patients showing typical B19V-induced erythema infectiousum or asymptomatic individuals were not investigated. A more targeted investigation with a high number of B19V-positive individuals is thus required to accurately describe current B19V epidemiological trends.

The identification of measles-suspected cases that tested positive for anti-MeV IgM at the admitting hospital but that were afterward discarded for both measles and rubella and found to be B19V DNA-positive is also an aspect that deserves particular attention. Sera from patients with other rash illnesses associated with B19V, rubella, and HHV-6 can yield anti-MeV IgM false-positive results and, particularly in settings of high measles vaccination coverage and low MeV prevalence, a high portion of IgM tests may be falsely positive for measles.[12,13] This highlights the crucial role of epidemiological investigations and differential diagnoses performed within the context of measles surveillance to confirm or disprove measles cases, especially in elimination settings.

In Italy, as in most EU/EEA member states, systematic surveillance of B19V and other exanthematous virus infections is not established. For this reason, there is substantial uncertainty regarding the real levels of circulation of B19V in the EU/EEA countries. Our study highlights that the "fever and rash" surveillance strategy not only is crucial in comprehensively monitoring measles and rubella cases, but it can also be easily adapted to monitor different epidemiological scenarios, such as the current one characterised by the co-circulation of MeV and B19V. In such a situation, active surveillance of B19V cases should be promoted, especially considering the potential risks of complications and hospitalisations associated with this virus.[9] Ideally, existing measles and rubella surveillance systems should be able to quickly extend the monitoring activities to other viruses, so that active surveillance can be implemented in case of rapidly changing epidemiological scenarios.

AUTHOR CONTRIBUTIONS

Conceptualisation, SB and AA; investigation, CF, MG, SB, SS, DCo, MF, DCe and LV; data curation, CF, MG, SB, and MC; writing—original draft preparation, CF, MG, SB, MC and AA; contributed analysis: MG, CF, SB, DCo, and MC; project administration, AA; supervision, MC and AA; resources, DCe; funding acquisition, AA; writing—review and editing, DCe, LV, and AA. All authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request. All the available data are included in the manuscript.

ETHICS STATEMENT

Ethical review and approval and patient consent were waived for this study as it was carried out as part of the Integrated Measles-Rubella Surveillance, performed by law in accordance with the Prime Minister's Decree of 3 March 2017 (https://www.gazzettaufficiale.it/eli/id/2017/05/12/17A03142/sg).

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