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ORIGINAL ARTICLE

Etiological analysis of discarded measles in the context of a measles outbreak among a highly immunized population

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Abstract

Background: Measles can lead to serious complications and remains an important cause of morbidity and mortality worldwide. In this study we aimed to assess the etiological diagnosis of discarded measles cases in the context of an outbreak among a highly immunized population.

Methods: We conducted a retrospective observational study of discarded measles cases from an outbreak that occurred from October 2006 to July 2007 in Catalonia. A confirmed case was defined as having a positive measles serum IgM result and/or a positive result by RT-PCR in urine and/or nasopharyngeal swab; or an epidemiological link to a confirmed case. Serum specimens were tested by a commercially available indirect-format and by an in-house capture-format measles IgM enzyme immunoassays.

Results: Testing of 89 samples discarded for measles determined the etiologies for 10 (11.2%), including one rubella, three human herpes virus 6, and six measles infections. Of 381 confirmed cases in the outbreak, 10% had received at least one dose of the measles-mumps-rubella vaccine versus 54% of the discarded for measles (OR: 0.09; 95% CI: 0.06, 0.14; $p < 0.001$).

Conclusions: Highly sensitive surveillance systems are critical to identifying cases, responding to outbreaks and verifying progress towards measles elimination. Molecular tools for measles detection and differential diagnosis, and collection of appropriate specimens for molecular and serological testing are essential to correctly diagnose suspected measles infection.

KEYWORDS
differential diagnosis, elimination, measles virus, surveillance, viral exanthemas

INTRODUCTION

Measles is a highly contagious viral disease that can lead to serious complications and remains an important cause of disease worldwide. Global efforts to achieve elimination with a safe and effective vaccine have reduced cases from 853,479 in 2000 to 360,296 in 2018, preventing more than 20 million deaths since 2000.[1,2] Multiple countries have interrupted endemic transmission of measles and sustained elimination while others have not been able to sustain elimination. In Europe, thousands of cases among all age groups were reported from July 2019 to July 2020, mainly in Romania (1284 cases), Bulgaria (257 cases), France (219 cases) and Germany (131 cases).[3] Despite this, the substantial decline in measles cases reported in Europe

and worldwide after March 2020 and through 2021 might be related to the COVID-19 pandemic; this decline might be explained by under-reporting or underdiagnosis, although transmission barriers could also have had an effect.[2] Low case counts were reported in 2021 in Germany (63 cases) and in Poland (14 cases). The withdrawal of nonpharmaceutical interventions could lead to an up rise of measles outbreaks.[4] To verify the elimination of measles, rubella and congenital rubella syndrome, the World Health Organization (WHO) has established that a country must have a high-quality surveillance system and demonstrate 36 months with no cases of endemic transmission.[5]

In Spain, elimination of measles was achieved in 2016[6,7] and, at a regional level, Catalonia certified autochthonous measles elimination in 2000. Elimination status in Catalonia has been maintained since then despite the occurrence of outbreaks of imported origin.[8-10]

Measles should always be considered in any person presenting with fever and erythematous rash.[11] Suspected measles cases should be isolated, and adequate clinical samples for laboratory confirmation should be collected. Contact tracing should be initiated immediately to avoid further transmission. Initial diagnosis is based on symptoms but epidemiological features such as travel and vaccination history, previous contact with a suspected or confirmed measles case or having attended an emergency room ward are also relevant. The diagnostic criterion of choice is the detection of specific IgM antibodies in sera drawn within 3–28 days of rash onset, yet other criteria such as measles-specific IgG seroconversion in two samples separated by an interval of 2–4 weeks or detection of measles virus (MeV) nucleic acid in a clinical specimen are also valid.[12] Yet, early confirmation by detection of MeV in urine or pharyngeal swab by reverse transcription polymerase chain reaction (RT-PCR) within 8 days following rash onset is needed whenever possible because it allows for prompt confirmation and genotyping of positive samples to trace transmission chains in outbreaks.

Once a clinically-compatible illness tests negative for MeV-infection (discarded), a differential diagnosis to further assure the illness is not erroneously classified as a nonmeasles infection is desirable in elimination settings although not mandatory. Differential diagnosis includes other viruses causing exanthema such as rubella virus, human herpes virus 6, cytomegalovirus, parvovirus B19, Epstein–Barr virus, varicella virus and arboviral diseases (dengue, zika and chikungunya) as well as conditions such as Kawasaki disease especially in children.[13,14]

The aim of this study was to assess the etiological diagnosis of discarded suspected measles infections ruled out by testing that were registered in Catalonia in the context of an outbreak among a highly immunized population.[8]

METHODS

A retrospective observational study of suspected measles infections ruled out by laboratory testing reported during an outbreak from October 2006 to July 2007 in Catalonia was conducted.[8] A suspected measles case was defined as a case with maculopapular rash, fever (>38°C), and cough, conjunctivitis, and/or coryza. A confirmed case was defined as a case with laboratory confirmation (positive measles IgM result in serum and/or a positive result for measles RNA by RT-PCR in urine and/or nasopharyngeal swab) or epidemiological link to a laboratory confirmed case. A discarded case was defined as an illness that meets the clinical criteria for measles but with a negative IgM result and without an epidemiological link to a confirmed measles case.[12]

Blood samples were collected 0–14 days after rash onset and were tested at the Hospital Clinic (Barcelona), the support laboratory of the Measles Elimination Plan in Catalonia, using Vircell Measles IgM ELISA (Granada, Spain), an indirect-format enzyme immunoassay that is reported to be 98% specific and 100% sensitive by the manufacturer. Urine specimens were collected ≤10 days after rash onset, frozen at −80°C and shipped to the National Microbiology Center of the Carlos III Health Institute (Madrid) where multiplex-nested RT-PCR was performed to detect measles, rubella, and parvovirus B19 viruses.[15]

According to the guidelines for measles surveillance of the Spanish National Epidemiological Surveillance Network (RENAVE: Red Nacional de Vigilancia Epidemiológica), discarded cases of measles should be tested for rubella, and if they are also negative for rubella, rule out at least parvovirus B19 infection.[12] Serum specimens of discarded cases were stored in the Hospital Clinic laboratory at −20°C until shipped to the Viral Vaccine Preventable Diseases Branch of the US Centers for Disease Control and Prevention (CDC) (Atlanta, GA) in 2014, where they were tested using the enzyme immunoassays (EIA) indicated in Table 1. All commercial EIAs were performed following instructions from the manufacturers. In-house measles capture IgM EIA, in-house measles IgG avidity EIA and plaque reduction neutralization (PRN) assay were performed as previously described. The IgM assay with a capture-format was developed at the United States Centers for Disease Control and Prevention (CDC) and it was found to be >99% specific and 100% sensitive by Hummel et al.[16,17,18,19] Human herpes virus 6 (HHV6) IgG testing was performed on specimens from children <2 years old since by this age, children have already had a primary infection with HHV6.[20,21] Specimens with HHV6 IgG negative results were then tested for HHV6 IgM.

Elevated measles IgG titers in previously vaccinated individuals have been associated with secondary vaccine failures.[22,23] To further examine this phenomenon, discarded cases that tested positive by the measles capture

TABLE 1 Enzyme immunoassays used to test samples from discarded measles cases. Catalonia 2006–2007 outbreak

Agent	Enzyme immunoassays	Positive result	Equivocal result	Negative result	Format
MeV	Trinity Captia measles IgG	ISR ≥1.10	0.91–1.09	ISR ≤ 0.90	Indirect
	In-house CDC measles IgM	P-N ≥ 0.100 and P/N ≥ 3.00	P-N ≥ 0.100 or P/N ≥ 3.00	P-N < 0.100 and P/N < 3.00	Capture
RuV	Zeus (Wampole) rubella IgG	Index value ≥1.10	0.91–1.09	Index value ≤0.90	Indirect
	Diamedix rubella IgM	Index value ≥1.10	0.90–1.09	Index value <0.90	Capture
Parvovirus B19	Biotrin B19 IgG	Index value >1.1	0.9–1.1	Index value <0.9	Indirect
	Biotrin B19 IgM	Index value >1.1	0.9–1.1	Index value <0.9	Capture
HHV6	Abnova HHV6 IgG	Index value >1.10	0.90–1.10	Index value <0.90	Indirect
	Viditest HHV6 IgM	Index value >1.10	0.90–1.10	Index value <0.9	Indirect
Agent	**Enzyme Immunoassays**	**High avidity**	**Intermediate avidity**	**Low avidity**	**Format**
MeV	In-house CDC measles IgG avidity	≥70%	30%–70%	≤30%	Indirect
RuV	Zeus-based rubella IgG avidity	≥30%	n/a	<30%	Indirect
Agent	**Combined assays**	**Confirmation of case with laboratory evidence of PE to MeV**			**Format**
MeV	Plaque reduction neutralization and IgG avidity	Concentration ≥40,000 mIU/ml and high avidity result			n/a

Note: P, average of optical density signal in wells coated with measles virus nucleoprotein antigen; N, average of optical density signal in wells coated with uninfected cell control antigen.
Abbreviations: MeV, measles virus; PE, previous exposure; RuV, Rubella Virus.

IgM assay, and had high avidity IgG and an IgG immune status ratio (ISR) >4.5 were tested by the PRN assay; PRN titers ≥40,000 mIU/ml were considered indicative of an anamnestic immune response. This provided confirmation that the IgM result was truly positive.[18] Confirmed cases were classified as measles breakthrough cases (confirmed measles cases with documented vaccination) further classified as primary vaccine failures (low avidity result) or secondary vaccine failures (high avidity result). Confirmed measles cases that were reported to be unvaccinated or with unknown vaccination status were classified as confirmed cases with laboratory evidence of previous exposure (PE) to MeV.

Differences were assessed by Chi square test and Fisher's exact test with 95% confidence intervals (95% CI) and odds ratio (OR). Vaccine effectiveness (VE) was calculated as 1-OR. Statistical significance was set at alpha error = 0.05.

Ethical considerations

All data used in the analysis were collected during routine public health surveillance activities, as part of the legislated mandate of the Health Department of Catalonia, the competent authority for the surveillance of communicable diseases, which is officially authorized to receive, treat and temporarily store personal data on cases of infectious disease. Therefore, data were exempt from Institutional Board Review and did not require informed consent. All data were rendered anonymous and gathered through public health outbreak surveillance activities. The CDC Human Research Protections Office determined that this study was not human subjects' research and not subject to CDC Institutional Review Board review.

RESULTS

Of the 538 suspected measles cases reported during the study period, 381 (70.8%) were laboratory confirmed or had a direct epidemiologic link to a measles laboratory confirmed case. Of these confirmed cases, only 10% (38/381) were vaccinated, whereas 54% (85/157) of the 157 discarded cases had been vaccinated with at least one dose of measles, mumps and rubella (MMR) vaccine (OR: 0.09; 95% CI: 0.06–0.14; p <0.001). Vaccine effectiveness observed in the context of the outbreak cohort was 91% (95% CI: 86%–94%).

Among the 85 vaccinated, discarded measles, 83 presented with classic measles symptoms, while two presented with rash and fever only. The median age of confirmed measles cases was 15 months (range: 1 month–50 years) and for discarded cases was 18 months (range: 3 months–48 years).

Discarded cases with a sufficient quantity of serum to perform all required serology tests (n = 89) were analyzed for confirmation and differential diagnosis of rubella, parvovirus B19 and, in children <2 years of age, HHV6 (Figure 1). Of the 89 discarded cases tested, 40 were unvaccinated, 30 had received one MMR dose, 18 had received two doses of MMR, and vaccination status was unknown for one case. Testing determined the etiologies for 11.2% (10/89) of the specimens and identified one rubella, three HHV6, and six measles infections. An etiological agent could not be determined for 79 samples (Table 2).

Measles

Six measles cases were identified by a measles-specific capture IgM assay and were classified as three cases, two breakthrough cases (one primary vaccine failure and one secondary vaccine failure), and one case with laboratory evidence of PE to MeV. At the time of the outbreak, four of the six samples were measles IgM negative by an indirect measles assay performed in the local laboratory. We identified the three measles cases in unvaccinated children (11–22 months old) based on results that were measles IgG negative and IgM positive by the capture assay. We also identified the case with laboratory evidence of PE to MeV based on a high avidity IgG result and a titer of 69,952 mIU/ml (ISR = 4.9); this case was measles IgM positive (capture format). Serological analysis of two specimens with measles IgM positive results by the indirect and capture assays identified a secondary vaccine failure case (high avidity result and titer of 91,106 mIU/ml; ISR = 5.0) and a primary vaccine failure case (low avidity result). Measles IgM was detected in seven additional specimens using the capture assay, of which three were collected from individuals who had received their first MMR dose approximately 3 weeks before sample collection (one was identified as an HHV6 case). Four of the seven had measles high avidity results (ISR <4.5) and were classified as undetermined cases with false positive IgM results.

HHV6

HHV6 IgM was detected in three samples. One of these three samples was collected from an 18-month-old child who had received the MMR vaccine 21 days prior to blood collection and had measles and rubella IgM positive and low avidity IgG results consistent with recent MMR vaccination.

Rubella

Rubella IgM was detected in four samples, which were classified as one rubella infection in an unvaccinated 8-year-old child with a rubella IgG negative result and

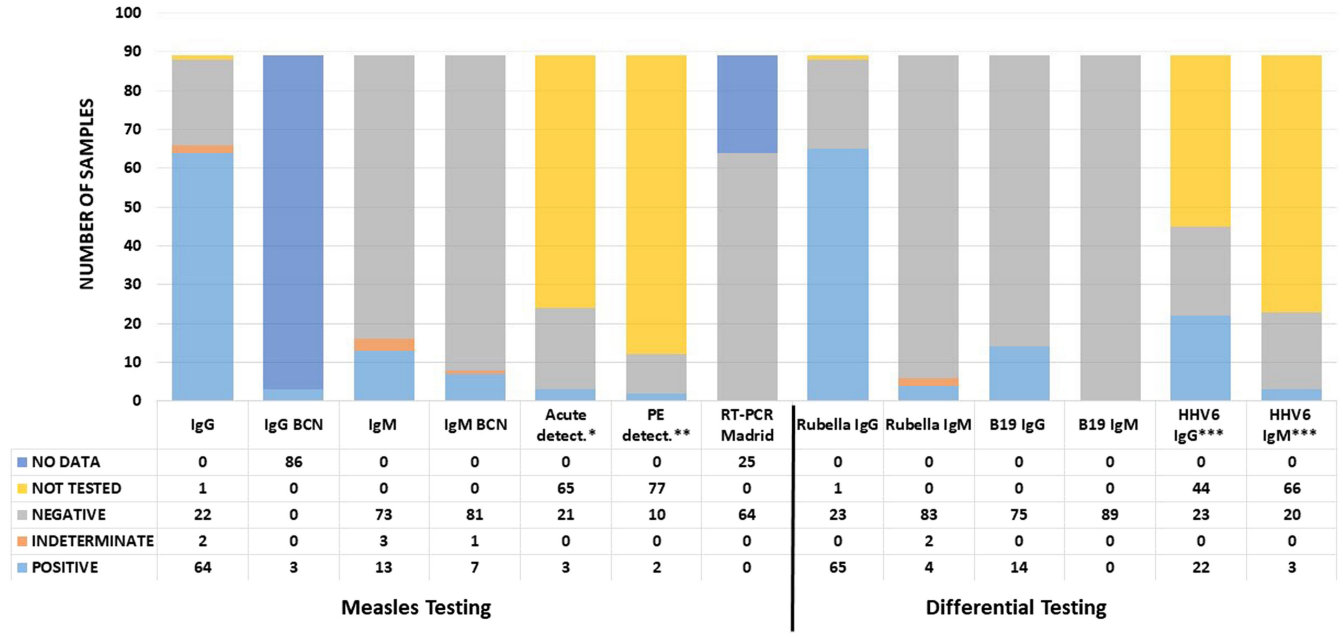

FIGURE 1 Distribution of results of discarded measles cases. Catalonia, 2006–2007. B19, Parvovirus B19; BCN, Hospital Clinic laboratory; HHV6, human herpes virus 6; RT-PCR, Reverse transcription polymerase chain reaction. Madrid: Centro Nacional Microbiología, Reference laboratory. Madrid. *Acute detection: positive if measles IgG avidity is low. **PE or previous exposure: positive if plaque reduction neutralization (PRN) titer >40,000 mIU/ml and IgG avidity is high; negative if PRN titer ≤40,000 mIU/ml and IgG avidity is high. ***Only children <2 years were tested for HHV6 IgG. Only children with an HHV6 IgG negative result were tested for HHV6 IgM.

TABLE 2 Etiologic investigation of discarded measles cases from a community outbreak. Catalonia 2006–2007

Etiology	Number of cases	% of total discarded cases with available sample
Determined	10	11.2
HHV6	3	3
MeV	3	3
Measles breakthrough[a]	2	2
Primary vaccine failure	1	
Secondary vaccine failure	1	
Measles with laboratory evidence of previous exposure to MeV	1	1
Parvovirus B19	0	0
RuV	1	1
Undetermined	79	89
Other etiology	76	85
Quantity not sufficient	1	1
Recent MMR immunization	2	2
Total	89	100

Abbreviations: HHV6, Human herpes virus 6; MeV, measles virus; MMR, measles, mumps and rubella; RuV, Rubella virus.

[a]Measles breakthrough: Confirmed measles case in an individual with documented measles vaccination. Breakthrough cases are classified into (1) primary vaccine failure (failure to mount a protective measles-specific immune response to the vaccine) and (2) secondary vaccine failures (decline in measles-vaccine immune response to insufficient levels for measles protection).

measles IgG and IgM negative results. The patient had no travel history yet belonged to a foreign family and might have had unknown contact with an imported case. Two IgM positive samples were from patients with recent MMR vaccination, with samples collected approximately 3 weeks after MMR and had low avidity IgG for both measles and rubella. The remaining case was a suspected cross-reaction with a rubella IgM false positive result in an individual identified as a measles secondary vaccine failure.

Parvovirus B19

All 89 specimens had a negative IgM result for parvovirus B19.

DISCUSSION

The WHO European Region has steadily progressed towards the elimination of measles with half of the 51 Member States interrupting endemic transmission. Despite this progress, there are challenges in surveillance still to be addressed such as determining immunity gaps in specific populations and identifying cases correctly through highly sensitive surveillance supported by laboratory networks. Enhanced surveillance could allow for rapid outbreak detection and control by confirming measles cases and providing genomic sequence data.[24] In elimination settings, where every case of measles must be properly identified, public health interventions depend on having highly sensitive and specific diagnostic tools.[25] Type of specimen collected and timing of sampling as well as proper specimen handling are also important contributors to this high-quality system.[26] Clinically compatible measles cases for which available information (laboratory results, epidemiological data and vaccination history) is sufficient to make an alternative diagnosis can be discarded as non-measles cases. Suspected measles cases cannot be confirmed based on clinical information only. Therefore, if there are no positive laboratory test results of suitable specimens nor epidemiological linkage to a laboratory confirmed case, an alternative diagnosis is required.[27]

This was a retrospective study focused on an outbreak occurring in a highly immunized population and when laboratory confirmation of cases was mainly based on IgM testing of suspected measles cases. In this study, only 11.2% of the tested samples from discarded measles rendered an etiologic confirmation, and of these, six out of 10 (60%) with etiology were found to be true measles cases. Of the six suspected cases that were ruled out in 2006, five were not tested for measles-specific IgG at the time. The use of measles high-avidity results and PRN titers ≥40,000 mIU/ml to help identify infections in vaccinated individuals was not recognized until 2016 by Sowers et al.[18] An evaluation of the frequency of false negative IgM results cannot be provided given the fact that all six suspected measles cases were reclassified in this study using MeV IgG, avidity and PRN testing, which were not used in 2006. High-performance diagnostic assays and a comprehensive algorithm of laboratory results that includes testing for MeV IgG, IgM and RNA, with MeV IgG avidity and PRN assays, when required, are measures that can assist in measles surveillance in elimination settings when analyzed together with clinical and epidemiological information.[25]

The clinical case definition for measles has a fever component which was firmly adhered to when classifying the cases, therefore discarded cases may be due to other illnesses, infectious and noninfectious, that present with maculopapular rash, fever and at least one of cough, coryza or conjunctivitis.[28,29] A limitation of our study was that the differential diagnosis performed was not exhaustive. We only tested for measles, rubella, parvovirus B19 and HHV6. It is possible that the agents responsible for the remaining rash and fever illnesses were not included in our testing. Another limitation was that we tested the specimens several years after being collected.[30] Freeze–thaw cycles of the specimens could potentially have detrimental effects on IgM stability, resulting in IgM negative results.[30] Misclassifying cases can lead to further transmission if preventive measures have not been applied and highlights the importance of collecting both serum and molecular samples for serological and RT-PCR testing for accurate case classification and gold standard surveillance.[5] Our analysis of combined IgG and IgM results was valuable in the identification of three acute measles cases and emphasizes the need to conduct serological testing for measles IgG in elimination settings. Advanced serological analysis including testing for measles IgG avidity and neutralizing antibodies was valuable in the identification of additional two breakthrough measles cases and one case with PE to MeV (all IgM positive per the capture format assay). As shown here and elsewhere, detection of elevated neutralizing titers in high-avidity IgG samples and detection of low-avidity IgG antibodies in the absence of recent measles vaccination can assist in identifying some cases.[31-33] Testing for avidity of the IgG antibody response helps discriminate between primary and secondary vaccine failure in highly immunized populations and this was used here to classify the two identified vaccine failures.[5,18]

As RT-PCR testing has become a widespread tool for measles confirmation in Catalonia, serological testing has diminished and there is scarcity of serum samples to perform serological tests for differential diagnosis with other exanthema causing viruses. Thus, the advantage of only collecting specimens for RT-PCR testing to provide timely confirmation, genotyping and sequencing of MeV hampers the assessment of specific IgM and IgG antibody features because of the concomitant lack of serum samples.

Characterization of the immune response is becoming more and more relevant to assess possible waning of protective immunity derived from the vaccine in an elimination scenario with scarce MeV circulation. Such characterization will become more relevant for future guidelines in adult populations at higher risk of being infected, such as healthcare personnel with two previous doses of measles-containing vaccine. Outbreaks in healthcare facilities with a growing proportion of immunized cases illustrates the importance of assessing how efficient immunity to MeV is in the long-term.[6,34-36]

Vaccination is the most effective measure to prevent measles and curtail outbreaks,[37] with two doses of measles containing vaccine being ~97% effective at preventing measles and one dose ~93% effective.[5] Vaccine effectiveness observed (91%) in this outbreak cohort was at the expected level due to median age of the cohort

being <18 months and, consequently, only one dose had been administered.

Highly sensitive surveillance systems are critical for identifying cases, responding to outbreaks and verifying progress towards elimination.[24] Complementary tools for differential diagnosis at the molecular level to screen for other viruses and stressing the collection of blood samples for additional testing should be considered essential in practice to follow MeV elimination guidelines at national and local levels.

AUTHOR CONTRIBUTIONS

All the authors participated in the study design, implementation and interpretation. N.T., S.M., J.C. and A.D. designed the study and drafted the report and conducted the laboratory and statistical analysis. A.M., S.B.S., E.S.A., W.J.B., and C.J.H. supervised the study, and reviewed the draft report. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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