Detection of Measles Virus RNA in Air and Surface Specimens in a Hospital Setting

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Measles virus (MeV) is known to be highly contagious, with an infectious period lasting from 4 days before to 4 days after rash onset. An unvaccinated, young, female patient with measles confirmed by direct epidemiologic link was hospitalized on day 5 after rash onset. Environmental samples were collected over the 4-day period of hospitalization in a single room. MeV RNA was detectable in air specimens, on surface specimens, and on respirators on days 5–8 after rash onset. This is the first report of environmental surveillance for MeV, and the results suggest that MeV-infected fomites may be present in healthcare settings.

**Keywords.** measles; aerosol; exposure; infectious period; transmission; infection prevention.

Measles is one of the most contagious viral infections known to infect humans [1]. The disease is caused by measles virus (MeV), a single-stranded, negative-sense, enveloped RNA virus of the genus *Morbillivirus* within the family *Paramyxoviridae*. Measles is a systemic infection characterized by fever, a maculopapular rash, cough, coryza, and conjunctivitis. The incubation period ranges from 8 to 14 days, with an average of approximately 10 days. Measles cases are infectious from 4 days before rash onset until 4 days after rash onset, with the rash generally occurring around 14 days after infection [1]. MeV and MeV RNA can be routinely detected in clinical samples collected >7 days after rash onset [2], and MeV RNA can be detected in samples from patients with measles for up to 3 months, especially in cases of immunosuppression [3, 4].

Nosocomial transmission of measles is frequent. However, to date, the presence of MeV in the environment occupied by a MeV-infected patient has not been studied directly. Obtaining these data can assist in explaining the highly contagious nature of the virus and may guide the refinement of infection prevention measures. This is the first report of the environmental burden of MeV detected in the air, on surfaces, and on respirators used by healthcare providers (HCPs) during routine care of a patient hospitalized with measles.

**METHODS**

**Patient Information**
An unvaccinated, otherwise healthy, immunocompetent young woman developed extreme fatigue and fever 11 days after exposure to a known measles case [5]. On day 13 after exposure, a diffuse rash started on the trunk and face, eventually spreading to extremities. She was admitted to the hospital to rule out pneumonia on day 18 after exposure, 5 days after onset of rash. She received measles, mumps, and rubella vaccine 4 days after exposure to the known case. The patient was placed on contact and airborne isolation during hospitalization. Patient activities such as movement and coughing were noted by study personnel. The Wake Forest Baptist Medical Center institutional review board waived approval requirements owing to the study’s limited focus on environmental sampling.

**Setting**
Wake Forest Baptist Medical Center is an 885-bed tertiary-care teaching hospital. Sampling was conducted in a single negative-pressure isolation room under turbulent airflow (6 total air changes/hour specified) at approximately 20°C and 40% relative humidity. The room underwent daily cleaning of high-touch areas.

**MeV Sampling**
Daily air samples were collected using 6-stage Andersen air sampling devices at 3 locations: the head of the bed/chair, 2 feet away from patient head; the middle of the bed/chair, 4 feet away from the head; and the foot of the bed/chair, 8 feet away from head [6]. The samples were collected in viral transport medium (VTM), aliquoted, and either added to AVL buffer with carrier RNA (Qiagen) or directly frozen at −80°C.

To test for MeV on environmental surfaces, sterile swabs were moistened with VTM, and a 6.45-cm² area was swabbed once at 3 high-touch locations daily: the head of the bed handrail, the middle of the food tray table, and a table at the back of the room (approximately 3 m from the foot of the bed). The swabs were vortexed for 30 seconds and processed as described for air samples.

Respirators (N-95) worn by HCPs were collected daily, and a 12.9-cm² midsection of the respirator was cut out, using sterile scissors. The cutouts were placed in 2 mL of VTM and frozen.
After thawing, samples were vortexed/agitated and further processed as described for air samples.

**RNA Sample Preparation**

RNA extraction was performed on samples containing AVL, using the QIAmp Viral RNA Mini Extraction Kit (catalog no. 52 906; Qiagen) per the manufacturer’s instructions. To increase sample detection, the samples were concentrated by extracting 3 replicates of each sample and loading them onto 1 QIAmp Mini column.

**Real-Time Quantitative Reverse-Transcription Polymerase Chain Reaction (qRT-PCR) Analysis**

Real-time qRT-PCR analysis was used to detect MeV in samples, using primers targeting the nucleoprotein gene [7]. RNA extracted from samples (2.5 µL) was used in each 25 µL reaction. Real-time qRT-PCR reactions were performed in duplicate, using the SuperScript III Platinum One-Step qRT-PCR kit (catalog no. 11732-020; Life Technologies) on the ABI 7500 real-time instrument for 40 cycles.

Samples were considered positive for MeV if the threshold cycle (Ct) value for both duplicates was <38 and negative if no Ct value (denoting an undetermined result) was produced. Samples that had discordant duplicates (Ct values that differed by >1.5 or in which 1 duplicate had a negative result), and samples that had Ct values of 38–40 were considered to have indeterminate results and were repeated. Positive samples were subjected to sequence analysis [8].

**Tissue Culture Infectivity Testing**

Vero/hSLAM cells were inoculated with 0.5 mL of sample and maintained in Dulbecco’s modified Eagle’s medium supplemented with 2% fetal bovine serum, 100 µ/mL penicillin, 100 µg/mL streptomycin, and 0.4 mg/mL G418 sulfate [9]. Cells were observed by light microscopy on a daily basis to look for cytopathic effect. Three passages of infected cells were performed. If no cytopathic effect was observed after 3 passages, the sample was considered negative.

**RESULTS**

The patient spent the first 48 hours mostly in bed sleeping, followed by being alert and sitting up in bed during sampling. She had minor coughing episodes on day 5, which intensified on days 6 and 7 after rash onset.

MeV RNA was detected in aerosol samples on days 5 (420 MeV RNA copies/10-L respiratory volume/minute; 1 positive sample) and 7 (1517 MeV RNA copies/10-L respiratory volume/minute; 3 positive samples) after rash onset at all sampling locations. Particle size distribution changed with distance to the patient’s head, with particles >4.7 µm detected only close to the patient’s head and small particles <4.7 µm detected at the middle and foot of the bed (Figure 1).

Surface samples tested positive for MeV RNA on all 4 study days, with higher copy numbers on days 6 and 7 after rash onset (Figure 2). The bed rail at the head of the bed showed the highest average copy number, with 564736 MeV RNA copies/cm² (4 positive samples), followed by the bedside table (13 MeV RNA copies/cm²; 1 positive sample) and a second table 3 m away (1 MeV RNA copies/cm²; 1 positive sample).

Out of 134 samples from respirators, 4 (3%) tested positive for MeV RNA, all of which were worn on day 6 after rash onset. The RNA copy numbers ranged from 123 to 570 MeV RNA copies/cm², with an average of 333 MeV RNA copies/cm².

Sequencing was attempted on 8 of the positive samples, including all 6 positive surface swabs, the strongest positive air sample, and the strongest positive respirator sample. Sequences were obtained for 4 of these samples (the respirator sample and 3 surface samples), and all sequences were identical and members of measles genotype D8. These sequences were also identical to sequences obtained from clinical specimens collected.
During the outbreak in North Carolina [5]. Tissue culture infectivity testing did not detect MeV in a selection of PCR-positive samples.

**DISCUSSION**

Endemic measles transmission was declared in 2000 to have been eliminated in the United States, because of an effective vaccination program that has maintained very high coverage rates. In 2011, the Pan American Health Organization verified that surveillance parameters are adequate to ensure the maintenance of measles elimination in the United States [10]. However, measles is endemic in many parts of the world, and imported cases continue to cause outbreaks in the United States. Outbreaks are often associated with transmission in healthcare settings [11]. Understanding the risk of exposure to MeV could help to develop more-effective infection control procedures.

This study attempted to assess the environmental burden of MeV in a hospital setting during routine patient care. To our knowledge, this is the first study to directly detect evidence of airborne transmission of MeV.

Current Centers for Disease Control and Prevention guidelines for healthcare workers recommend airborne isolation for all patients suspected of having measles in healthcare settings [12]. The patient described in our report had a known epidemiologic link to a laboratory-confirmed measles case and displayed the classical symptoms; the patient was hospitalized 5 days after rash onset. Beginning on the first day of hospitalization, MeV RNA was detectable in the air up to 2.43 m from the patient, on surfaces within the hospital room, and on respirators worn by visitors and HCPs. MeV RNA detection patterns were associated with increased patient coughing and movement.

MeV RNA was detected up to 2.43 m from the patient, with larger droplets closer to the patient’s head and smaller droplet nuclei further away. Transmission via air has been associated with the explosive character of measles outbreaks, reflected in high basic reproductive rates of 15–20 [1]. Entry routes of the virus are thought to be through target cells in both alveolar spaces and the mucociliary epithelium of the mid and upper respiratory tract [13]. This makes transmission by exposure to the broad range of particle sizes and the long distance, as detected in this study, possible.

Transmission of MeV may occur not only through air but also through contact with surfaces in the direct-care environment of a patient. MeV survives on surfaces for <2 hours [14]. MeV RNA was detected in high concentrations on several high-touch areas, making transmission via indirect contact possible. Virus transfer through touching and settling of MeV-associated particles out of the air probably led to contamination of the areas in the close-care environment. However, MeV RNA was also detected on a rarely touched table 3 m from the patient, making deposition of MeV solely by air more likely.

Patient activities such as coughing have been associated with increased levels of influenza virus detection [6]. The patient with measles coughed more frequently during days 6 and 7, coinciding with the highest frequency of detection of viral RNA. Experimental infection of rhesus macaques showed large numbers of MeV-infected epithelial and lymphoid cells in the upper respiratory tract, and coughing and sneezing led to the shedding of MeV-infected cells, cell debris, and cell-free virus [15]. This suggests that there are at least 2 possible mechanisms of MeV transmission: from close contact with cell-associated virus and from cell-free MeV in small particles. Cell-associated transmission via large particles (>4.7 μm) may also be more effective than cell-free transmission, by providing greater protection against environmental hazards such as temperature, humidity, and UV radiation than cell-free transmission.
MeV RNA was also detected on N95 respirators worn by HCPs. Four of 134 masks tested positive for MeV RNA, matching the high level of air and surface detection observed on day 6. Testing was limited to the center portion of the respirator exposed to the air currents of the wearer. While contamination due to touching cannot be excluded, correct donning and removal usually do not expose the midsection of the mask. This novel detection method provides a direct assessment of the individual exposure to airborne viruses.

Of course, this study is limited to one patient, and shedding patterns are likely to vary considerably between patients. In this case, environmental testing was not initiated until day 5 after rash onset and did not occur during the postulated infectious period. Testing of PCR-positive samples in cell cultures failed to detect viable MeV, making it impossible to assess the potential of these samples to serve as a source of infection. However, these results show that environmental surveillance for MeV is possible and suggest that additional studies to measure shedding of MeV may provide insight into understanding the highly infectious nature of MeV.

Notes

Disclosure. 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. Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.