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Viral co-infections among children with confirmed measles at hospitals in Hanoi, Vietnam, 2014

Le Khanh Nguyen Hang, Loan Phuong Do, Thanh Thi Trieu Van, Son Vu Nguyen, Phuong Vu Mai Hoang, Hien Thi Pham, Thanh Thi Le, Huong Thi Thu Tran, Cuong Duc Vuong, Thi Quynh Le Mai✉

National Institute of Hygiene and Epidemiology, No 1-Yesrsin Street, Hanoi, Viet Nam

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ABSTRACT

Objective: To characterize viral co-infections among representative hospitalized measles cases during the 2014 Hanoi outbreak.
Methods: Throat swabs were collected from 54 pediatric patients with confirmed measles, and molecular diagnostics performed for 10 additional viral respiratory pathogens (Influenza A/H1N1pdm09; A/H3N2 and influenza B; Parainfluenza 1, 2, 3; Respiratory Synctial Virus, RSV; human Metapneumovirus, hMPV; Adenovirus and Picornavirus).
Results: Twenty-one cases (38.9%) showed evidence of infection with other respiratory viruses: 15 samples contained measles plus one additional virus, and 6 samples contained measles plus 2 additional viruses. Adenovirus was detected as a predominant cause of co-infections (13 cases; 24.1%), followed by RSV (6 cases; 11.1%), A/H1N1pdm09 (3 cases; 5.6%), PIV3 (3 cases; 3.7%), Rhinovirus (3 cases; 3.7%) and hMPV (1 case; 1.96%).
Conclusions: Viral co-infections identified from pediatric measles cases may have contributed to increased disease severity and high rate of fatal outcomes. Optimal treatment of measles cases may require control of multiple viral respiratory pathogens.

1. Introduction

Measles is a vaccine-preventable respiratory viral illness of the Paramyxovirus family, associated with symptoms of high fever, runny nose, white spots in the mouth and a hallmark rash. Despite inclusion of measles within the national immunization programs of most countries worldwide, the disease remains a leading cause of death in young children worldwide [1].

In Vietnam, measles vaccination has been included in the National Expanded Program for Immunization (EPI) since 2001, for children from nine months to six years, and elimination targets were originally identified in 2009 for achievement in 2012 [2]. However, the disease reemerged during 2008–2010 with 7948 confirmed cases [3] and then again in May 2013, when cases were reported from 24 cities and provinces, including the major urban centers of Hanoi and Ho Chi Minh city. In 2014, Vietnam reported more than 3500 confirmed measles infections, a single hospital in Hanoi-the National Pediatric Hospital (NHP)-treated as many as 1280 measles patients, of which at least 100 cases were fatal [4].

Pneumonia is the most common severe complication of measles infection and accounts for most measles-associated death [5]. Pneumonia may be caused by measles virus alone, secondary viral infections with adenovirus or RSV, or secondary bacterial infections. Viral co-infections are known to cause increased disease severity and are a risk factor for respiratory failure [6,7]. The importance of viral respiratory co-infections during hospitalization is the challenge for infection control and rapid spread among patient rooms, which is particularly difficult under conditions of over-crowding during outbreaks.

During April 2014, the Vietnam Ministry of Health swiftly responded to the measles outbreak through mobilizing the health system to provide patient treatment and diagnosis, to verify vaccination records of school-age children, and to sponsor catch-up vaccination campaigns for children at risk. However, over-crowding conditions at major hospitals and treatment centers in Hanoi, particularly at NHP and Bach Mai hospital (BMH) were reported, with alarming increases in the number of severe pneumonia patients [4,6]. The comprehensive strategy for patient

First author: Hang Khanh Nguyen, National Institute of Hygiene and Epidemiology, No 1-Yesrsin Street, Hanoi, Viet Nam.
E-mail: nlkh@nihe.org.vn
✉Corresponding author: Mai Quynh Le, National Influenza Center-National Institute of Hygiene and Epidemiology, Hanoi, Viet Nam.
Tel: +84 912126283
Fax: +84 4 3 8210853
E-mails: lom9@hotmail.com, lom9@nihe.org.vn
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treatment involved triage of the most severe measles cases, particularly those with pneumonia. Determination of other microbiology-associated measles complications was performed to provide adequate treatment to reduce mortality and control measles infection in hospitals.

Our study reports descriptive data on viral etiologies associated with hospitalized measles cases presenting to two hospitals in Hanoi during the peak of outbreak case detections in April, 2014. This study was undertaken to determine the frequency of co-infecting viral pathogens, including molecular detection of ten common respiratory viruses.

2. Material and methods

2.1. Study population

During April 2014, we collected residual respiratory specimens (nasopharyngeal swabs, tracheal aspirates) for patients that had screened positive for measles by RT-PCR using standard protocols as previously described [8] in the clinical diagnostic laboratories of NHP and BMH. All samples were originated from inpatient wards (intensive care units) and were submitted to the virology department of the National Institute of Hygiene and Epidemiology (NIHE). In total, samples from 54 measles-confirmed cases with severe pneumonia were obtained (30 from NPH, 24 from BMH). The case definition of measles severe pneumonia was high fever (>38.5 °C), cough, shortness of breath, rash and fatigue.

2.2. Sample processing

Nucleic acids were extracted using Qiagen viral RNA Mini Kit (Qiagen CA, USA). For each sample, 140 μL of VTM were processed according to the manufacturer instructions, eluted in 60 μL of Qiagen AVE buffer. Real time RT-PCR were used to screen for influenza A and B through detection of *M* gene, followed by subtyping by HA-specific RT-PCR [1,9]. Subsequently, all samples negative for influenza were screened by real time RT-PCR for parainfluenza (PIV) 1, 2, 3; hRSV; hMPV; adenoviruses, and by conventional RT-PCR for detection of rhinovirus. All tests were performed according to standard procedures as currently performed within the national surveillance program for Severe Acute Respiratory Illness previously described [9–12].

3. Results

The measles cases included in this study originated from 54 children with a median age of 11.5 months (range from 2 months to 4 years old), including 20 children <9 months old (37.0%) and 34 children >9 months (63.0%). 38 of the cases were boys (70.4%) and 16 were girls (29.6%). Samples from twenty-nine cases were collected within 5 days of hospital admission, eighteen samples were collected from 6 to 10 d post-admission, and seven samples were collected after 11 or more days of hospitalization.

Of the 54 measles confirmed samples that were tested for 10 other respiratory viruses, 15 (27.8%) were confirmed positive for a single additional viral pathogen. These comprised 2 (3.7%) cases of influenza A/H1N1pdm09 viruses; 8 (14.8%) cases of adenovirus; 4 (7.4%) RSV and 1 (1.9%) rhinovirus (Table 1).

Table 1
Detection of multiple viral infections in measles confirmed cases with severe pneumonia in Hanoi, April 2014.

Co-infection	Respiratory viruses positive
One additional virus	15/54 (27.8%)
A/H1N1pdm09	2 (3.7%)
Adenovirus	8 (14.8%)
RSV	4 (7.4%)
Rhinovirus	1 (1.9%)
Two additional viruses	6/54 (11.1%)
Adeno + A/H1N1pdm09	1 (1.9%)
Adeno + hMPV	1 (1.9%)
Adeno + RSV	1 (1.9%)
Adeno + PIV3	2 (1.9%)
RSV + Rhino	1 (1.9%)
Total	21/54 (38.9%)

Six measles-confirmed cases (11.1%) were identified as having co-infection with two additional viral pathogens: among these, co-infections with adenovirus and A/H1N1pdm09/hMPV/RSV/PIV3 were determined for 5 patients and one patient was found co-infected with RSV and rhinovirus (Table 1). We did not find either influenza A/H3 nor influenza B, nor any cases of PIV1 and PIV2 among the measles-confirmed samples.

The proportion of measles cases co-infected with one or two other respiratory viruses were 38.9% (21 patients). Adenovirus was identified in thirteen samples (61.9%), including eight cases (38%) of single co-infection and five cases (23.8%) of double viral co-infections (Table 1, Figure 1).

The adenovirus was the most frequently co-infections, identified in 13 samples, with children aged (2–15) months, median age of 7.8 months. The second most frequent viral co-infection was RSV, identified in 6 samples (28.6%), with children aged (6–24) months, median age of 12.2 months, including 4 cases of single co-infection and 2 cases of double viral co-infection identified in older children (Figure 1). A/H1N1pdm09 co-infections identified in 3 samples, with children aged (9–18) months, median age of 12.0 months. Rhinovirus co-infections identified in 2 samples, with children aged (6–24) months, median age of 15.0 months. hMPV co-infections identified in 1 samples, with children aged 6 months. PIV3 co-infections identified in 2 samples, with children aged (7–15) months, median age of 11.0 months.

Among 18 measles cases determined to have one or two additional co-infecting viruses, 13 were collected from 6 to 10 d post hospital admission (72.2%), 4 samples in 29 were taken

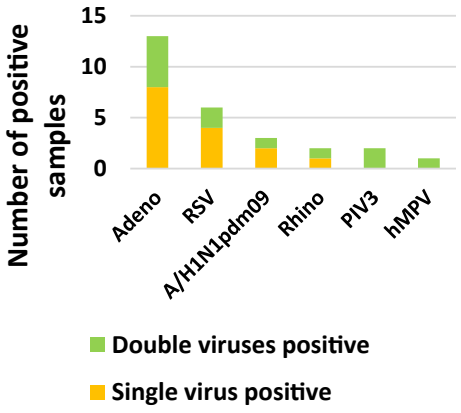

Figure 1. Incidence of respiratory viruses infections.

within 5 d of admission (13.8%) and other 4 in 7 samples were obtained at hospitalization day of 11 or longer (57.1%).

4. Discussion

Measles is a common childhood viral disease and remains one of the leading cause of deaths among children, particularly developing countries [13]. Pneumonia is major complication in hospitalized cases, occurring in about 60%–80% of cases with case fatality rates varying from 5% to 20% [14]. Pneumonia may be caused by measles alone, however co-infections with secondary viral infection or bacteria infections can increase severity and influence outcome. Viral co-infection in hospitalized pediatric patients with acute respiratory infections were documented more frequently when analyzing causes of acute pneumonia [15–17], however, our study is the first report of multiple viral co-infection following primary infection by measles virus in Vietnam. We detected viral co-infection in at least one third of children hospitalized with measles infection (21 in 54 cases; 38.9%). The age distribution of confirmed measles cases detected in this study showed 20 cases <9 months (*i.e.* among babies not yet scheduled for measles immunization), in contrast with 34 case detections among children >9 months. For the older children, vaccination status was difficult to confirm and often unknown, and case report forms from this outbreak showed that 86% may not have been immunized [4]. The measles outbreak in 2014 indicates critical short-comings in the implementation of national immunization programs in Vietnam, and the significant challenges ahead for meeting measles elimination targets by 2020 [2].

The viral co-infection rate detected in our study was 38.9%, including 27.8% with co-infections by a single virus and 11.1% by two additional viruses. This high rate of viral co-infection involved in severe pneumonia cases has not been previously reported from Vietnam, and strongly indicates that viral co-infections are a risk factor for disease severity.

A study in Shanghai in 2009–2010 reported 29.9% of cases with multiple viral co-infections among upper respiratory tract infections of children under three years old. Other studies from Spain and UK of hospitalized pediatric cases of lower respiratory tract infections have shown peaks of co-infections at (12–24) months of age, supporting our findings with a median age of co-infections at 11.5 months.

Our results showed adenovirus was the predominant co-infecting agent and rated 61.9% in total, consistent with autopsy examinations (postmortem biopsy) of measles cases who died in NPH during February–June 2014, showing adenovirus type 7 was a contributing cause of death in children with measles-associated immuno suppression. Adenovirus type 7 pneumonia should be recognized as a major cause of secondary infection after measles [6]. The second main cause of viral co-infection was RSV (6 samples; 28.6% in total) with age from (6–24) months, consistent with other studies showing RSV was the most frequent pathogen causing pneumonia in children under two years old [11,12,18]. We found a significant higher risk of viral co-infection among measles cases with long periods of hospitalization. The samples collected during the first 5 days of hospitalization showed lower rate of co-infection (13.8%) than samples collected later during hospital stay: 72.2% of samples collected from 6 to 10 days post-admission exhibited co-infections, as did 57.1% of samples collected after 11 days post-admission. The measles outbreak in 2014 led to extreme overcrowding in hospitals, especially NPH and BMH, thus our results reflected the poor conditions at that time. There is a continuing need for more hospital-based studies to evaluate intervention strategies to improve measles case management in Vietnam.

Our study had at least 3 limitations. First, our sample size was small since sampling was conducted only during April 2014 within two hospitals of Hanoi, and thus may not be representative for all measles hospitalized cases during the 2014 outbreak. Second, our study detected a broad range of common respiratory viral pathogens but testing was not exhaustive, and may have missed as yet underscribed respiratory pathogens. Lastly, the influence of concurrent bacterial infections was not analyzed in this study, and these are known to be important risk factors for severe pneumonia.

The presence of one or two additional respiratory viruses in measles confirmed cases admitted was determined for two hospitals in Hanoi, and highlighted the importance of adenovirus as the major cause of viral co-infections.

Increasing childhood vaccination and improving health facilities to reduce overcrowding in the hospitals are critical procedures to decrease co-infection rates leading to severe pneumonia and death among young children in Vietnam. Enhancing viral co-infection surveillance in hospitals to better understand viral etiologies of severe pediatric pneumonia may help to development of therapeutic and prevention strategies.

Conflict of interest statement

We declare that we have no conflict of interest.

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