Use of a multiplex polymerase chain reaction assay for the early detection of an outbreak of human parainfluenza virus type 3 infection in a nursery school during the COVID-19 pandemic

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SUMMARY

Introduction: Although outbreaks of parainfluenza virus type 3 (PIV-3) have been reported in children, to our knowledge none have been reported in a nursery school. As the symptoms of PIV-3 infection are similar to those of COVID-19 infection, accurate diagnosis of PIV-3 and other respiratory viruses is important during the COVID-19 pandemic.

Aims: We experienced an outbreak of upper respiratory symptoms at a nursery school in Miyagi Prefecture, Japan, from 29/5/2021 to 13/6/2021 and aimed to determine the causative organism(s).

Methods: A multiplex polymerase chain reaction (PCR) assay which enabled rapid detection of a variety of causative microorganisms of respiratory tract infections was used to analyse 13 nasopharyngeal swabs collected during the outbreak. Infection Prevention and control measures were implemented to prevent further spread of infection.

Results: All 13 samples were positive for PIV-3 infection. 2 of the 13 samples were also positive for rhinovirus/enterovirus and 1 sample was also positive for rhinovirus/enterovirus and coronavirus NL 63. No samples were positive for SARS-CoV-2.

Discussion: Children in school settings are especially vulnerable to respiratory viral infections, including COVID-19. Children under two years are unable to wear masks reliably, and the COVID-19 vaccine was approved only for older children. Multiplex PCR assays can be used for the rapid diagnosis of respiratory infections.
Introduction

Parainfluenza viruses (PIVs) belong to the Paramyxoviridae family and cause a variety of upper and lower respiratory tract illnesses [1]. Approximately 7% of all hospitalisations for fever and/or respiratory symptoms in children under 5 years are attributable to PIVs [2]. PIV type 3 (PIV-3) is the commonest of the four serotypes and outbreaks have been reported in neonatal intensive care units, haematological units (including stem cell transplantation units) and oncology wards [1,3–7]. To date no report has described an outbreak of PIV-3 infection in a nursery school.

In response to the current coronavirus disease 2019 (COVID-19) pandemic, the governments of several countries locked down cities and restricted the movement of people to prevent the spread of COVID-19 [8]. Early diagnosis of patients with respiratory symptoms is necessary to exclude COVID-19 and other serious illnesses and to enable early interventions, which can help to prevent further spread of the disease. The gold standard of diagnosis of COVID-19 is the direct detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA by nucleic acid amplification tests, most commonly reverse transcription polymerase chain reaction (RT-PCR), in samples from the upper respiratory tract. The COVID-19 pandemic may lead to more widespread use of RT-PCR for the diagnosis of respiratory infections [9]. A multiplex nested PCR assay (BioFire® Respiratory Panel 2.1, BioFire Diagnostics/bioMérieux, Salt Lake City, Utah, USA) that targets 18 viruses, including SARS-CoV-2, and 3 bacterial species was approved for use in Japan in October 2020. No multiplex PCR assay was available in Japan before the COVID-19 pandemic. We started using the test as soon as it was approved as it provides results in 45 minutes and therefore facilitates prompt diagnosis of multiple respiratory infections [10]. We report our experience using a multiplex nested PCR assay which detected an outbreak of PIV-3 infection in a nursery school in Miyagi Prefecture, Japan, in May/June 2021.

Case presentation

From 29/5/2021 to 13/6/2021, an outbreak of symptoms of an upper respiratory infection, including fever, cough, sputum production, and nasal discharge, occurred in 28 (approximately 97%) of the babies and children at a nursery school in Sendai, Miyagi Prefecture, Japan. Miyagi Prefecture is located in the northeastern part of Japan and has a population of about 2.31 million people. By September 2021, approximately 16,000 people in the prefecture had been infected with COVID-19.

The staff from the Institute of Infection Prevention and Control surveyed the nursery school and interviewed teachers and parents from 7/6/2021 to 14/6/2021. The data collected included age, gender, the onset and clinical course of the infection, the clinical outcome and the results of a nasopharyngeal swab tested using a multiplex nested PCR assay.

The nursery school

The nursery school is attached to the hospital and had 3 classes for 3 age groups of babies and children (under 1 year old, 1–2 years’ old, and 2 years old and older). It had 29 babies and children and 12 employees and is a full-time school that is open from Monday to Friday. Five employees worked during the day shift and two worked during the night shift, which was offered to babies and children of healthcare workers on Tuesdays and Fridays. At the time of the outbreak during the COVID-19 pandemic, all employees wore eye protection and surgical masks. They were required to take their temperatures daily, to record employees’ and children’s health daily and to practice good hand hygiene. All employees were fully vaccinated against COVID-19. It was difficult to ventilate the classrooms where the babies and children spent their time because, for safety reasons, the windows could only be opened a little and some rooms could not fulfil the requirements for the minimum number of air exchanges per hour. No large-scale communal activities had been held before the outbreak.

Multiplex nested PCR assay

To diagnose the cause(s) of the patients’ symptoms, the FilmArray®Respiratory Panel in the multiplex nested PCR assay BioFire®FilmArray® Torch System (bioMérieux, Marcyl’Etoile, France) was used in accordance with the manufacturer’s instructions. This assay targets 17 viruses, including SARS-CoV-2, and 3 bacterial species. Samples from 13 infected babies and children were tested to determine the presence of human adenovirus, various coronavirus (229E, HKU1, NL63, and OC43), human metapneumovirus, human rhinovirus/enterovirus, influenza virus A (H1, H1-2009, and H3) and B, human parainfluenza virus (types 1–4), respiratory syncytial virus, SARS-CoV-2 virus, Mycoplasma pneumoniae, Chlamydia pneumoniae, and Bordetella pertussis. We obtained the samples (at least 300 µl) by nasopharyngeal swab and injected them into the Viral Transport Medium in the FilmArray® pouch; the reaction proceeded automatically in the FilmArray® Torch system. Testing was performed only in babies and children whose parents consented; the parents of the 15 children and babies who were not tested did not provide consent because the babies’ and children’s symptoms had already improved, or the parents had taken their baby or child to another clinic.

Conclusion: We identified an outbreak of PIV-3 in a nursery school during the COVID-19 pandemic. The investigation of the outbreak highlighted that it was important not to overlook other respiratory infections including PIV-3 during the COVID-19 pandemic. The multiplex PCR assay provided rapid and accurate diagnosis of the causative organisms in the outbreak and helped to direct appropriate interventions to control the outbreak. © 2022 The Authors. Published by Elsevier Ltd on behalf of The Healthcare Infection Society. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
Results

Identification of the outbreak

Human PIV-3 infection was confirmed in all 13 samples (Table I). In addition, one sample was also positive for rhinovirus/enterovirus and coronavirus NL63 and two samples were also positive for rhinovirus/enterovirus. None of the samples were positive for SARS-CoV-2 virus. None of the babies or children in the nursery school was vaccinated against COVID-19.

Figure 1 compares the daily incidence of onset of PIV-3 symptoms in the babies and children with the trend of COVID-19 infection in Miyagi Prefecture during the same period.

Control of the outbreak

Following review by the Infection Prevention and Control team (IPCT) on 7/6/2021, the nursery school was closed and all the babies and children were required to be isolated at home. All of them recovered and returned to the nursery school 8 days after this intervention. In addition to the temporary closure of the nursery school the IPCT requested that the nursery school employees ensured that the classrooms were thoroughly ventilated, checked the physical condition of the babies and children at home (by contacting the parents) and at the nursery school and informed the parents not to bring their babies and children to the school if they were unwell. In addition, the following measures were introduced: improved hand hygiene among all staff and children; enhanced decontamination of the environment, such as increased frequency of cleaning and disinfection of toys; increased frequency of ventilation of each room and assessment of the ventilation efficiency in each room by measuring carbon dioxide levels with a sensor. After the introduction of these interventions, the PIV-3 outbreak ended and did not reoccur.

Discussion

In this study, we showed that multiplex nested PCR assay was useful for early detection of a PIV-3 outbreak in a nursery school during the COVID-19 pandemic.

In the COVID-19 pandemic, universal masking, improved hand hygiene, social distancing, and vaccination have been important steps to prevent the spread of infection [11]. However, not all of these steps can be undertaken by children. In Japan, as of October 2021, no COVID-19 vaccine had been approved for children younger than 12 years [12]. Consequently, the prompt diagnosis of respiratory viral infection in babies and children is essential.

Outbreaks of infections in schools, where the causative microorganism cannot be identified, are stressful for both school employees and parents, particularly during the COVID-19 pandemic. Most causes of respiratory infections in children are viral and were previously determined by viral culture. Therefore, in the past it was difficult to identify the causative microorganism of outbreaks in schools with poor access to facilities that can perform viral cultures (Japan has only a limited number of facilities that can culture viruses). If the causative microorganism of an outbreak cannot be identified,

| Table I: The results of multiplex PCR assays in thirteen patients in the nursery school 1. Patient number | Age (Year) | Sex (M/F) | Onset date (day) | Test date (day) | Results of multiplex PCR assays | Outcome |
|---|---|---|---|---|---|---|
| 1 | 1 | F | 7/6/2021 | 7/6/2021 | Parainfluenza virus type 3 | Recovered |
| 2 | 2 | F | 6/6/2021 | 7/6/2021 | Parainfluenza virus type 3 Rhinovirus/enterovirus Coronavirus NL63 | Recovered |
| 3 | 1 | M | 7/6/2021 | 7/6/2021 | Parainfluenza virus type 3 | Recovered |
| 4 | 0 | F | 3/6/2021 | 8/6/2021 | Parainfluenza virus type 3 | Recovered |
| 5 | 2 | F | 7/6/2021 | 8/6/2021 | Parainfluenza virus type 3 Rhinovirus/enterovirus | Recovered |
| 6 | 2 | F | 5/6/2021 | 8/6/2021 | Parainfluenza virus type 3 | Recovered |
| 7 | 2 | F | 6/6/2021 | 8/6/2021 | Parainfluenza virus type 3 | Recovered |
| 8 | 2 | F | 5/6/2021 | 8/6/2021 | Parainfluenza virus type 3 Rhinovirus/enterovirus | Recovered |
| 9 | 2 | M | 8/6/2021 | 9/6/2021 | Parainfluenza virus type 3 | Recovered |
| 10 | 1 | F | 7/6/2021 | 9/6/2021 | Parainfluenza virus type 3 | Recovered |
| 11 | 1 | F | 5/6/2021 | 10/6/2021 | Parainfluenza virus type 3 | Recovered |
| 12 | 2 | M | 13/6/2021 | 14/6/2021 | Parainfluenza virus type 3 | Recovered |
| 13 | 1 | M | 5/6/2021 | 14/6/2021 | Parainfluenza virus type 3 | Recovered |

M, male; F, Female; PCR, Polymerase Chain Reaction.
it is difficult to determine how long a school must remain closed.

The multiplex nested PCR assay was easy to perform and the results were ready within 45 minutes. Because PCR does not differentiate colonisation from infection, a positive result does not necessarily indicate infection. However, a previous study showed that positive test results for PIV-3 were more frequently detected in children with acute respiratory symptoms than in asymptomatic children [13]. In our study, all tested patients were symptomatic. Another study indicated that PCR detects PIV-3 at a higher rate than viral culture [14]. Thus, the multiplex nested PCR assay is a valuable tool in the COVID-19 pandemic because it allows rapid identification of a wide variety of causative microorganisms of respiratory tract infections.

The reason why the outbreak of PIV-3 occurred in the nursery school is unclear but it may have been caused by a lack of thorough infection control measures. In the COVID-19 pandemic, universal masking and ventilation tend to be the focus of infection control measures. However, fomites and contaminated surfaces are more important routes of PIV transmission than aerosols [15]. Consequently, in particular babies and very young children, who often put things in their mouths and may not have adequate hand hygiene, can be the origin of an outbreak. Once an outbreak occurs at a nursery school, it is difficult to end it without closing the school for a period of time. The current study suggests that in environments such as nursery schools attention should also be directed to decontamination of the environment and not just to droplet and airborne infection control.

In this study, the PIV-3 outbreak occurred in the spring. PIV-3 infection peaks in the spring and has a second, smaller peak in the fall [16].

This study has some limitations. Firstly we did not follow up the multiplex nested PCR assays with cultures. Secondly, our survey was conducted only in those babies and children whose parents gave consent. Thirdly, we did not test the babies’ and children’s parents who had upper respiratory symptoms because their symptoms were mild, and they did not wish to be tested. Fourthly, nursery school staff were not tested because they were asymptomatic, so it is unclear how widespread the PIV-3 outbreak was. Lastly we could not clearly identify the source and mode of transmission of PIV-3 in the nursery school, which may have represented a bias in the results.

In conclusion, we reported on an outbreak of PIV-3 in a nursery school in Miyagi Prefecture, Japan, in May/June 2021. A key message from the outbreak was that it is important not to overlook other infections, including PIV-3, during the COVID-19 pandemic. A diagnostic approach using multiplex nested PCR assay provided rapid accurate diagnosis of the causative organisms in respiratory infections and helped to direct appropriate interventions to control the outbreak.

Authors’ contributions

Jun Suzuki: Writing - Original draft preparation, Methodology, Investigation; Shiro Endo: Writing - Review & Editing, Conceptualization, Investigation; Tomoki Mizuno: Investigation, Shota Takahashi: Data Curation; Yukiko Horiuchi: Data Curation; Yuri Ami: Data Curation; Haruka Imai: Investigation; Daishi Shimada: Investigation; Makiko Yoshida: Investigation, Data Curation; Mitsuo Kaku: Funding acquisition; Masafumi Seki: Supervision.
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Ethics approval and consent to participate

The study was approved by the Institutional Review Board of Tohoku Medical and Pharmaceutical University Hospital (2021-2-087).

Conflict of interest

All authors declare that they have no competing interests.

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