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Short report

Molecular epidemiology of an outbreak of human parainfluenza virus 3 among oncology patients

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SUMMARY

A hospital outbreak of human parainfluenza virus type 3 (HPIV-3) in haematologic oncology patients is described in 12 patients over a four-week period. Exposure histories and molecular analysis of HPIV-3 isolates suggest that both community-acquired and nosocomially transmitted infections occurred during this outbreak. Molecular analysis of HPIV-3 isolates indicated that a chain of transmission occurred among multiple patients in an oncology ward. This transmission was later determined to be associated with the movement of fomites, visitors, and activities in the unit. The infection prevention team stopped nosocomial spread of HPIV-3 through interventions including advanced cleaning procedures.

Introduction

Human parainfluenza virus type 3 (HPIV-3) causes upper and lower respiratory illnesses most commonly in the early spring and summer months. The incubation period for parainfluenza is two to seven days [1,2]. Among immunocompromised patients, HPIV is an important cause of morbidity and mortality [3–5]. Hospital outbreaks of HPIV require prompt identification and mitigation efforts to prevent transmission events [6]. Several features of HPIV including its stability in the environment and ability to be spread by large-droplet aerosols and fomites make adherence to infection prevention measures essential in preventing HPIV transmission events [7]. The use of rapid molecular diagnostic respiratory virus panels has greatly aided hospital infection control efforts, as they support rapid identification of new infections and initiation of appropriate isolation precautions. This report describes an HPIV-3 outbreak

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among hospitalized haematologic oncology patients and the mitigation strategies used to stop the outbreak.

Methods

Hospital setting and patient population

Duke University Hospital (DUH) is a large academic teaching hospital with 957 inpatient beds. DUH provides care for a large number of immunocompromised patients including high-acuity oncology, critical care, trauma, solid organ and bone marrow transplant patients. In early June 2017, Duke Infection Prevention and Hospital Epidemiology identified four patients with HPIV-3, all residing on the same 31-bed haematological malignancy unit.

Investigation and mitigation

The infection prevention team reviewed the medical records of all HPIV-3-infected patients to identify any epidemiologic links including overlap in space and time on the unit, exposure to common healthcare providers, and exposure to other patients who were hospitalized with community-acquired HPIV-3. Healthcare providers who provided direct patient care were interviewed to identify the presence of any respiratory viral symptoms such as cough, sore throat, fever, or rhinorrhoea. No providers reported respiratory symptoms. Three additional patients with community-acquired HPIV-3 infection diagnosed one month previous were identified and had overlapping admissions on the same unit, suggesting possible healthcare-associated transmission.

The infection prevention team performed daily rounds on the unit and re-educated unit staff on the importance of basic infection prevention measures including: compliance with contact precautions, use of dedicated equipment whenever possible, meticulous cleaning of shared equipment, hand hygiene, and not working when sick. In addition, a request was placed for environmental services to deep clean all common spaces on the unit using bleach. Furthermore, infection prevention and the unit manager performed additional audits of compliance with hand hygiene and contact precautions.

Despite these measures, seven additional cases of HPIV-3 occurred during the subsequent two weeks on the same unit. Infection prevention recommended enhanced cleaning measures to continue until the outbreak subsided including: (i) the use of adjunctive UVC light (TRU-D) (BioRad, Hercules, CA, USA) for all discharge cleans, (ii) curtain changes with all discharge cleans, (iii) bleach cleaning for all cleaning activities on the unit, and (iv) increased audits of room cleanliness using ultraviolet markers. In addition, droplet precautions were paired with the recommended contact precautions for all parainfluenza-positive patients and for any patients being admitted with respiratory symptoms. Finally, recreational therapy was restricted (e.g. group therapy, crafts, pet therapy, hospitality carts, and volunteer visits). Additional investigation revealed that a spouse of one of the HPIV-3-infected patients was making hand-woven scarves, hats, and other personal items for other patients on the unit. Furthermore, a candy dispenser was found at the unit clerk desk. Patient families and visitors were subsequently restricted from visiting one another and sharing personal items and the candy dispenser was decommissioned.

Over the next 20 days the infection prevention team continued to test any patients with respiratory symptoms and to isolate patients who previously tested positive for parainfluenza within the past month upon subsequent readmissions. The team continued to see positive test results from patients known to be infected but no additional healthcare-associated cases were identified. At this point, recreational therapy and pet therapy were reinstituted and volunteers were again allowed to visit the unit. The outbreak was declared to be over in early July 2017.

Sample collection

Nasopharyngeal (NP) swab samples were collected as a part of routine clinical practice from patients with respiratory viral infection symptoms. NP swab samples were tested for respiratory viruses including HPIV types 1–4, influenza A and B virus, respiratory syncytial virus, human metapneumovirus, and rhinovirus with multiplex polymerase chain reaction (PCR) (GenMark, Carlsbad, CA, USA). When HPIV-3 infection was diagnosed, NP samples for HPIV-3 testing were taken afterward for each patient every week until negative or until the patient was discharged.

Real-time reverse transcription PCR and HPIV-3 strain sequencing

Viral RNA was extracted from human clinical samples using the QIAamp Viral RNA Mini Kit (Qiagen, Inc., Valencia, CA, USA). The respiratory specimens from 12 patients with HPIV-3 infections were included in the post-outbreak epidemiological investigation, involving partial genome sequence analysis of the haemagglutinin–neuraminidase (HN) and fusion protein (F) gene. As a positive control reference sequence, HPIV-3 was obtained through BEI Resources, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MA, USA: NIH 47885, NR-323.

Reverse transcription PCR was performed on the extracts using the HN and fusion gene primers described by Kim *et al.* [8]. The Invitrogen SuperScript III One-Step RT-PCR System with Platinum Taq DNA Polymerase kit (Cat. no.: 12574026; Thermo Fisher Scientific Inc., Waltham, MA, USA) was used according to the manufacturer's instructions. Briefly, 5 μL of RNA template was amplified in a 25 μL reaction mixture containing 2× one-step reaction buffer, 1 μL of SuperScript III Platinum Taq mix, and primers at a final concentration of 0.25 μM. Thermal cycling conditions were: 30 min at 55°C, 2 min at 94°C, followed by 45 cycles of 15 s at 94°C, 30 s at 50°C, and 1 min at 68°C, followed by a final elongation step for 5 min at 68°C. A positive PCR result was confirmed by agarose gel electrophoresis and UV illumination using a BioRad Gel Doc EZ Imager (Bio-Rad).

Sequences were aligned with MegAlign 15 software (DNAStar, Madison, WI, USA) using the Clustal W method. Phylogenetic analysis was performed using the MegAlign 15 software maximum likelihood method with a bootstrap value of 1000. Fusion gene sequencing was used to differentiate the epidemiological links between an equivocal cluster identified during HN gene sequencing.

Results

Human HPIV-3 outbreak characteristics

From May 10th, 2017 to July 3rd, 2017, 12 patients with HPIV-3 were identified on a 31-bed haematological malignancy unit (Figure 1). All patients had a haematological malignancy, most frequently leukaemia, and no patients had viral co-infections.

During the outbreak, the infection prevention team discovered that patients' family members and visitors had become friends with one another during their loved-one's extended stays in the hospital. Family members were found visiting each other in patient rooms and common spaces and were also found to be sharing personal items. In addition, one HPIV-3 infected patient's spouse was making scarves and hats and donating them to other patients and family members on the unit including many of the patients who developed HPIV-3 infection. In total, there were 12 individual positive patients with HPIV-3, nine of which were determined to be healthcare-associated. Four patients died; however, their deaths were attributed to their cancer diagnosis, not to HPIV-3 infection. HPIV was thought to be directly and indirectly transmitted via patient and family interactions, fomites, and infrequent cleaning of common spaces.

Molecular analysis

A phylogenetic tree (Figure 2) of HPIV-3 HN and fusion gene sequences from 12 molecularly confirmed outbreak cases and a BEI control HPIV-3 strain (NR-323). The HN gene phylogenetic tree demonstrated a major cluster (cluster 1, Figure 2) of 10 outbreak cases. Further analysis of the F gene within cluster 1 was performed because the HN gene had low discriminatory power within cluster 1. The F gene analysis revealed four sub-clusters of two identical HPIV-3 strains (relevant to cases 1 and 10; 7 and 9; 6 and 11; 5 and 13).

Discussion

There are multiple reports of HPIV-3 outbreaks occurring among immunocompromised patients due to either a single strain or due to multiple strains circulating in the community [9–12]. In our analysis, sequencing and phylogenetic analysis of HPIV-3 strains helped to determine whether single or multiple strains were responsible for the hospital outbreak. Although some reports indicate that infection control measures may be ineffective in limiting the transmission of HPIV-3, the present study underscores the importance of early intervention in reducing the number of additional outbreak cases [6].

Our data demonstrated that an HPIV-3 outbreak in a haematological malignancy unit caused considerable morbidity in nine out of 12 patients infected. Based on our phylogenetic analysis, two sub-clusters of identical HPIV-3 strains were circulating in the patients. Our team identified multiple potential pathways of transmission, one of which was the sharing of items (scarves and hats) among patients and family members. These findings provide important evidence for probable routes for nosocomial HPIV-3 transmission and emphasize the importance of adherence to infection prevention and control practices. Additionally, these findings underscore the importance of tracking visitation during periods of high HPIV-3 in the community.

Previous studies have used molecular techniques to evaluate the spread of HPIV-3 outbreaks, both nosocomial and in community-acquired hospital infections [9,12–14]. In general, it is thought that the epidemiology of HPIV-3 in inpatients mirrors that of the outpatient population [15]; however, there is growing evidence that nosocomial infections may play a more important role than community-imported HPIV-3

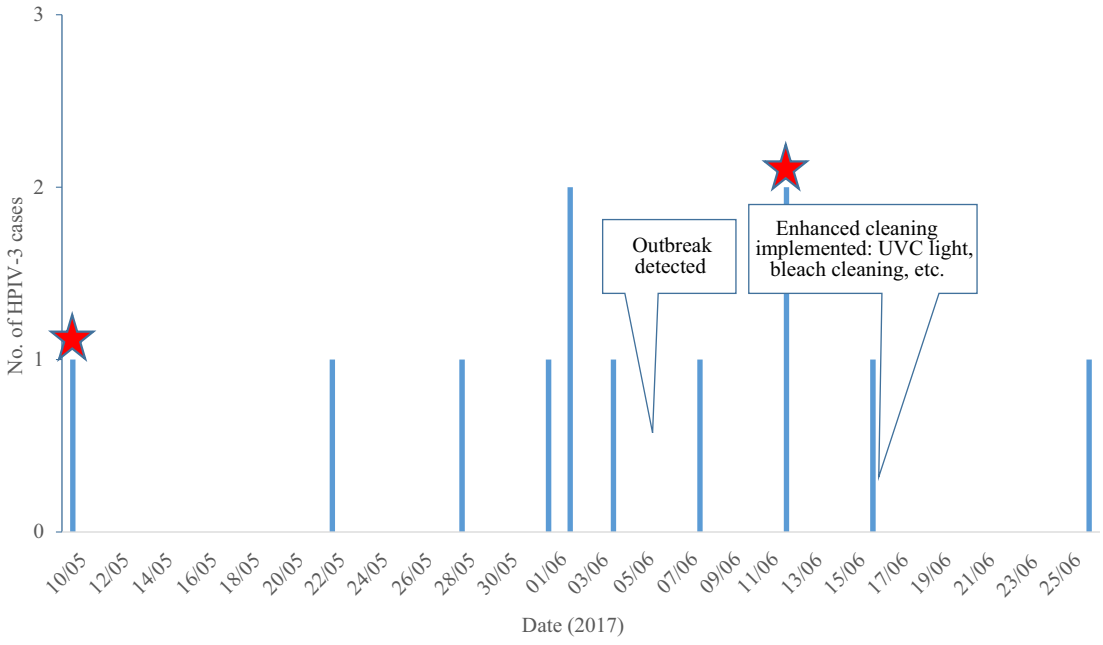

Figure 1. Number of human parainfluenza virus type 3 (HPIV-3) cases detected during the outbreak. Red stars indicate community-acquired cases.

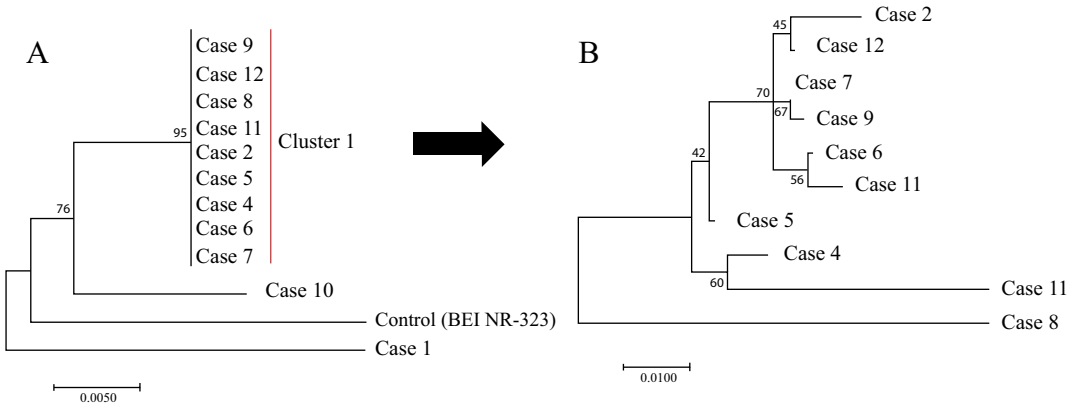

Figure 2. Phylogenetic analysis of the 12 molecularly identified and one control human parainfluenza virus type 3 (HPIV-3) strains. The scale indicates the relative genetic distance between each patient sample. (A) Sequence comparison of HPIV-3 sequenced in the haemagglutinin–neuraminidase (HN) gene of 450 bp. (B) Sequence comparison of HPIV-3 sequence in the fusion protein (F) gene from cluster 1.

[6,9,11–13,16]. In this study, we found that infections acquired and spread within the hospital played the greatest role in the spread of HPIV-3 through the patient population.

As HPIV is associated with high levels of morbidity and mortality in immunocompromised hosts, it is important to note that there were no deaths in our outbreak group directly related to HPIV-3 infection. This is fortunate as previous reports indicate that the mortality rate of HPIV-3 ranges from 3% to 47% according to various patient characteristics (transplant type, immunosuppression therapy, etc.) [11,13,16,17]. In this outbreak, a multimodal approach of strict infection control procedures greatly reduced the spread of HPIV-3 within the patient population and between patients and family members. Specifically, re-education on transmission dynamics, enhanced isolation practices (droplet + contact), enhanced cleaning procedures, increased audits of compliance with infection prevention practices (hand hygiene, cleaning, and isolation practices), and the restriction of group therapy crafts mitigated the outbreak.

This study has important limitations. First, samples detected by RT-PCR may not contain infectious virus; however, our data provide insight into the identity of the respiratory virus affecting this cohort of patients with haematological malignancies which aided in treatment and prevention of additional cases. Second, in the phylogenetic analysis of the HN gene, clusters of cases were grouped together with a small number of nucleotide (1–3) bp differences. As such, we analysed the F gene, another variable region, for this cluster in order to increase our discriminatory ability.

In conclusion, this study suggests that the spread of HPIV-3 through the hospital can be limited through effective infection prevention and control practices. In this patient population, the spread of HPIV-3 may have been through social interactions of patients and family members who shared group activities as well as scarves and hats among each other. The findings of this study reinforce the premise that preventive measures should include isolation of infected patients, meticulous cleaning practices, and appropriately educating healthcare providers, patients, family, and visitors on infection prevention practices.

Conflict of interest statement
None declared.

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