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An Outbreak of Human Parainfluenza Virus 3 (Phylogenetic Subcluster C5) Infection among Adults at a Residential Care Facility for the Disabled in Croatia, 2018

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Keywords
Human parainfluenza virus 3 · Pneumonia · Community residential care home · Multiplex PCR · Sequencing

Abstract
Introduction: Although highly pertinent for children, outbreaks of human parainfluenza virus (HPIV) may cause up to 15% of all respiratory illnesses in adults and predispose them to serious adverse outcomes, with HPIV serotype 3 (HPIV3) being the most common. This study represents the first report of an HPIV3 outbreak among adults at a long-term health-care facility in Croatia. ***Methods:*** A retrospective study was conducted to investigate an outbreak of acute respiratory infection (ARI) at a single residential care facility for the disabled in Croatia. Demographic, epidemiological, and clinical data were collected for all residents, while hospitalized patients were appraised in detail by laboratory/radiological methods. Multiplex PCR for respiratory viruses and sequencing was performed. Partial HPIV3 HN 581 nt sequences were aligned with HPIV3 sequences from the GenBank database to conduct a phylogenetic analysis, where different bioinformatic approaches were employed. ***Results:*** In late June 2018, 5 of the 10 units at the facility were affected by the outbreak. Among the 106 residents, 23 (21.7%) developed ARI, and 6 (26.1%) of them were hospitalized. HPIV3 was identified in 18 (73%) of the residents and 5 (83%) of the hospitalized individuals. Isolated HPIV3 strains were classified within the phylogenetic subcluster C5 but grouped on 2 separate branches of the phylogenetic tree. During the entire outbreak period, none of the institution's employees reported symptoms of ARI. ***Conclusions:*** Our study has shown that this health care-associated outbreak of HPIV3 infection could have been linked to multiple importation events. Preventive measures in curbing such incidents should be enforced vigorously.

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Introduction

Human parainfluenza viruses (HPIVs) 1–4 belong to the family Paramyxoviridae and are divided into 2 genera, *Respirovirus* (serotype 1 and serotype 3) and *Rubulavirus* (serotype 2 and serotype 4). All of them are known to cause upper and lower respiratory tract infections [1, 2]. Although HPIV epidemics are responsible for a significant burden of disease in children (accounting for approximately 40% of all pediatric hospitalizations due to lower respiratory tract infections), they may also cause up to 15% of all respiratory illnesses in adults and predispose debilitated and/or frail older individuals to severe disease [3, 4].

Infections caused by HPIV may be sporadic, although outbreaks are frequent. Seasonal variations in the occurrence of HPIV infections are generally seen when serotype-specific rates of infection are analyzed, which are highly dependent on the region [3–5]. More specifically, the seasonal infection patterns observed in the northern hemisphere, in countries with temperate climate, are basically absent in tropical and subtropical regions, where there is little variation in infection rates during the year [3, 6].

HPIV serotype 3 (HPIV3) represents the most commonly detected serotype in both children and adults presenting with symptomatic disease [3, 5], which is also the case in Croatia [7, 8]. In a recent phylogenetic analysis, the HPIV3 cluster C/subcluster C3 (genetic lineage C3a) was found to be the most frequent circulating HPIV3 strain among Croatian children with respiratory diseases [8]. However, the significance of other types, especially HPIV serotype 1, in epidemic years should be emphasized as well [3, 9, 10].

Outbreaks of HPIV in health-care settings have been primarily described in neonatal units [11–13] and hematology/oncology wards [14–16]. Moreover, HPIV3 has been found to account for 90% of the nosocomial infections in bone marrow transplant units during peak viral seasons [3, 17–19]. Additionally, HPIV infection has also been documented in elderly individuals residing in long-term care facilities, with prospective studies describing 4–14% annual infection rates [3].

In healthy adults, HPIV infection typically presents as a mild, self-limited upper respiratory tract illness with symptoms such as cough, rhinorrhea, and sore throat (with or without fever) [1, 3]. However, in predisposed individuals, infection may give rise to severe disease, lung function deterioration, as well prolonged hospitalization necessitating intensive care and even mechanical ventilation [1, 3, 17, 19]. Therefore, early recognition of HPIV outbreaks among hospitalized patients or residents of long-term care facilities with various risk factors is pivotal.

This study represents the first published description of an HPIV outbreak among adults situated at a long-term health-care setting (residential care facility) in Croatia. We also emphasize the importance of viral sequencing and phylogenetic analysis in our approach. The outbreak was discovered after a prompt and comprehensive evaluation of the etiological, epidemiological, and clinical specificities of an acute respiratory illness observed among the residents of a rehabilitation facility for persons with disabilities.

Materials and Methods

Patients and Specimens

A retrospective study was conducted to investigate an outbreak of acute respiratory infection (ARI) at the Stančić Residential Care Facility in Dugo Selo, Croatia, which provides residential and rehabilitation services to children and adults with physical, intellectual, sensory, and/or mental disabilities. A total of 203 residents resided at the facility in the pavilion for nonambulatory patients, divided into 10 separate units. Both medical and nonmedical personnel were assigned to care for the residents in each unit. A total of 23 cases of ARI were identified at the facility between June 22 and July 3, 2018. Pharyngeal and nasopharyngeal swabs were collected, placed in viral transport media, and subsequently analyzed using multiplex polymerase chain reaction (PCR) to establish the etiological diagnosis. Demographic, epidemiological, and clinical data were collected for all the residents, while laboratory and radiological data were only collected for hospitalized patients. In addition, blood cultures and the urine *Legionella* antigen test were performed for the patients hospitalized with pneumonia to determine the etiology of their illness.

Laboratory Testing

Multiplex PCR for the Detection of Respiratory Viruses

To isolate viral nucleic acid from viral transport medium (UTM™, Copan, Italy), the QIAamp® MinElute® Virus Spin Kit (Qiagen, Hilden, Germany) was used following the manufacturer's protocol. Multiplex PCR and cDNA synthesis were performed in a one-step reaction using a Seeplex® RV15 Detection Kit (Seegene Inc., Seoul, Korea) on a GeneAmp® 9700 PCR System Thermal Cycler (Applied Biosystems, Foster City, CA, USA), followed by microchip electrophoresis detection on an MCE®-202 MultiNA device (Shimadzu, Kyoto, Japan). Multiplex PCR was used to detect the following respiratory viruses: adenovirus, coronavirus 229E/NL63 and OC43, PIV types 1, 2, 3, and 4, influenza virus types A and B, respiratory syncytial virus types A and B, metapneumovirus, bocavirus, rhinovirus, and enterovirus [7].

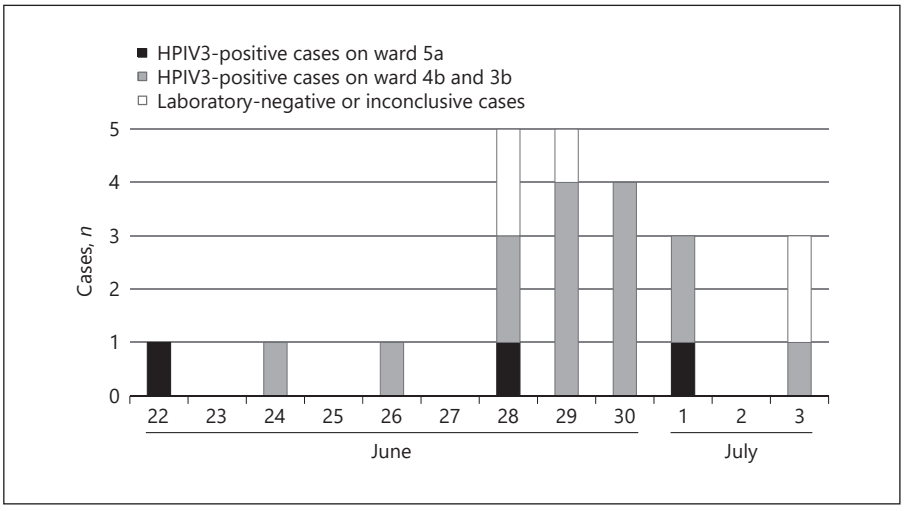

Fig. 1. Cases of acute respiratory infection in the facility by date of symptom onset, Croatia, June to July 2018 ($n = 23$). HPIV3, human parainfluenza virus type 3.

RNA Isolation, RT-PCR, and Sequencing

The method for obtaining HPIV3 HN partial 581 nt sequences from the clinical samples was previously described by Košutić-Gulija et al. [8]. Briefly, viral RNA was extracted from 250 μL of an NPA specimen, as reported previously by Chomczynski and Sacchi [20]. The cDNA was prepared from the total RNA using random hexamers and MuLV reverse transcriptase. Amplification was carried out with 10 μL cDNA, OneTaq DNA Polymerase (New England Biolabs), and specific primers for the HPIV3 HN gene (HN2 7494For and HPIV3 HN2 8697Rev). The 581 nt PCR products were visualized on 1.0% agarose gel, excised, and purified.

Purified PCR products were then sequenced by employing a Big Dye Terminator v3.1 Cycle Sequencing Kit on an automated DNA sequencer, ABI PRISM 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

Phylogenetic Analysis

For phylogenetic analysis, the 581 nt HN gene sequences obtained in this study were aligned with HPIV3 sequences downloaded from the GenBank database, which had been previously used in epidemiological studies.

The HN gene nucleotide sequences from this study were checked for similarity to database sequences using the Basic Local Alignment Search Tool (BLAST; http://blast.ncbi.nlm.nih.gov/Blast.cgi). Alignments were performed using ClustalX 2.1 software [21], and the selection of the most suitable substitution model was determined with jModelTest 2.1.4 software [22]. Bayesian Markov Chain Monte Carlo (MCMC) inference was performed with BEAST v1.8.2 [23]. Convergence was assessed based on the effective sample size using Tracer v1.5 (http://beast.bio.ed.ac.uk/Tracer) after a 10% burn-in, and only values above 200 were accepted. Maximum clade credibility trees were generated with TreeAnnotator v1.8.2 and visualized with FigTree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree).

The HPIV3 sequences obtained in this study were submitted to the GenBank under accession numbers MH684358–MH684367.

Results

In late June 2018, an outbreak occurred in 5 of the 10 units at the facility. Among the 106 residents in the units, 23 (21.7%) developed ARI. The cases of ARI at the facility are presented in Figure 1 according to the date of symptom onset. The average age of those infected was 46 years (range 22–73), of whom 19 (82.6%) were male.

All the patients presented with fever, muscle aches, fatigue, and upper respiratory tract symptoms. Seventeen of the 23 patients had only mild symptoms, were treated symptomatically, and did not require hospitalization.

Six of the 23 (26.1%) patients were hospitalized at the Dr. Fran Mihaljevic University Hospital for Infectious Diseases in Zagreb due to the severity of their illness. All six of these patients were motor impaired, suffered from epilepsy, and were on anticonvulsants, antipsychotics, and anxiolytics. None of them had other comorbidities or confirmed immunodeficiencies, and none was currently on immunosuppressants. All had a history of frequent respiratory infections.

The hospitalized patients had been admitted on average on the third day of illness (range 1–5). Mean C-reactive protein value on admission was 88 mg/L (range 15–132), and mean white blood cell count was 7,100 (range 4,600–10,500) cells per microliter. Other routine laboratory findings were within the limits of reference values. Chest radiographs were obtained in all the patients, which revealed interstitial patchy confluent infiltrates, unilateral in two patients and bilateral in four. Two of the patients also had small pleural effusions. The patients with pneumonia were treated symptomatically (antipyretics, anal-

Table 1. Clinical and laboratory data of 23 cases of acute respiratory infection at the Stancic Residential Care Facility from June 22 to July 3, 2018

Case No.	Clinical presentation	Unit	Hospital admission	Multiplex PCR	RT-PCR and sequencing	Sample identification number/gene accession number	Number of different nucleotides according to the strain MH684358 (sample 448) marked with x
1	pneumonia	4b	yes	PIV3	positive	441/**	0
2	pneumonia	4b	yes	PIV3	positive	442/**	0
3	pneumonia	4b	yes	PIV3	positive	444/MH684364	1
4	pneumonia	4b	yes	PIV3	positive	445/**	0
5	pneumonia	4b	yes	PIV3	positive	446/**	0
6	pneumonia	4b	yes	ND	ND	NA/NA	NA
7	URTI	3b	no	PIV3	positive	447/MH684360	2
8	URTI	4b	no	PIV3	positive	448/MH684358	x
9	URTI	3b	no	negative	ND	449/NA	NA
10	URTI	4b	no	PIV3	positive	450/**	0
11	URTI	4b	no	negative	ND	451/NA	NA
12	URTI	1b	no	inconclusive	ND	452/NA	NA
13	URTI	5a	no	PIV3	positive	453/MH684359	3
14	URTI	4b	no	PIV3	positive	454/**	0
15	URTI	4b	no	PIV3	positive	455/MH684363	2
16	URTI	4b	no	PIV3	positive	456/**	0
17	URTI	4b	no	PIV3	negative	457/NA	NA
18	URTI	4b	no	PIV3	positive	458/MH684361	2
19	URTI	4b	no	PIV3	positive	459/MH684365	4
20	URTI	6	no	negative	ND	460/NA	NA
21	URTI	4b	no	PIV3	positive	461/MH684362	1
22	URTI	5a	no	PIV3	positive	462/MH684366	5
23	URTI	5a	no	PIV3	positive	463/MH684367	3

URTI, upper respiratory tract infection; PIV3, parainfluenza virus type 3; NA, not applicable; ND, not done. ** Sequences are identical to those of strain MH684358 (sample 448).

gesics, crystalloid solutions, oxygen therapy with a face mask), and due to suspected bacterial pneumonia, all of them received empirical antimicrobial treatment: three a combination of co-amoxiclav and azithromycin, one a combination of piperacillin/tazobactam and azithromycin, and two levofloxacin as a monotherapy. None of the patients required intensive care or mechanical ventilation.

In all 23 patients, the illness lasted for an average of 9.7 days (range 4–28). All had good outcomes. The hospitalization of the pneumonia patients lasted for a mean of 7.3 days (range 4–11).

The hospitalized patients with pneumonia underwent a routine workup to determine the etiology of their disease: blood cultures, rapid urine *Legionella* antigen test, and serological screening for atypical pathogens (*Mycoplasma pneumoniae*, *Chlamydophila pneumoniae*, *Chlamydophila psittaci*, *Legionella pneumophila*, and *Coxiella burnetii*). None of these bacterial pathogens was identified as the cause of the pneumonia. Further testing of nasopharyngeal and pharyngeal swabs using the multiplex PCR method identified HPIV3 in 18/23 (73%) of the residents and 5/6 of the hospitalized patients by testing. No virus was detected in the specimens from 3 of the patients, the test results could not be interpreted for 1 patient, and no specimen was collected from 1 patient (Table 1). During the entire outbreak, none of the facility's employees reported symptoms of ARI. An epidemiological investigation failed to determine the potential source of the outbreak.

Partial HN gene sequences were successfully sequenced, and phylogenetic analysis has been performed from 17 clinical samples (Fig. 2). Seven strains showed identical sequences, represented by strain HR/27.18(448) MH684358 on the phylogenetic tree. The remaining

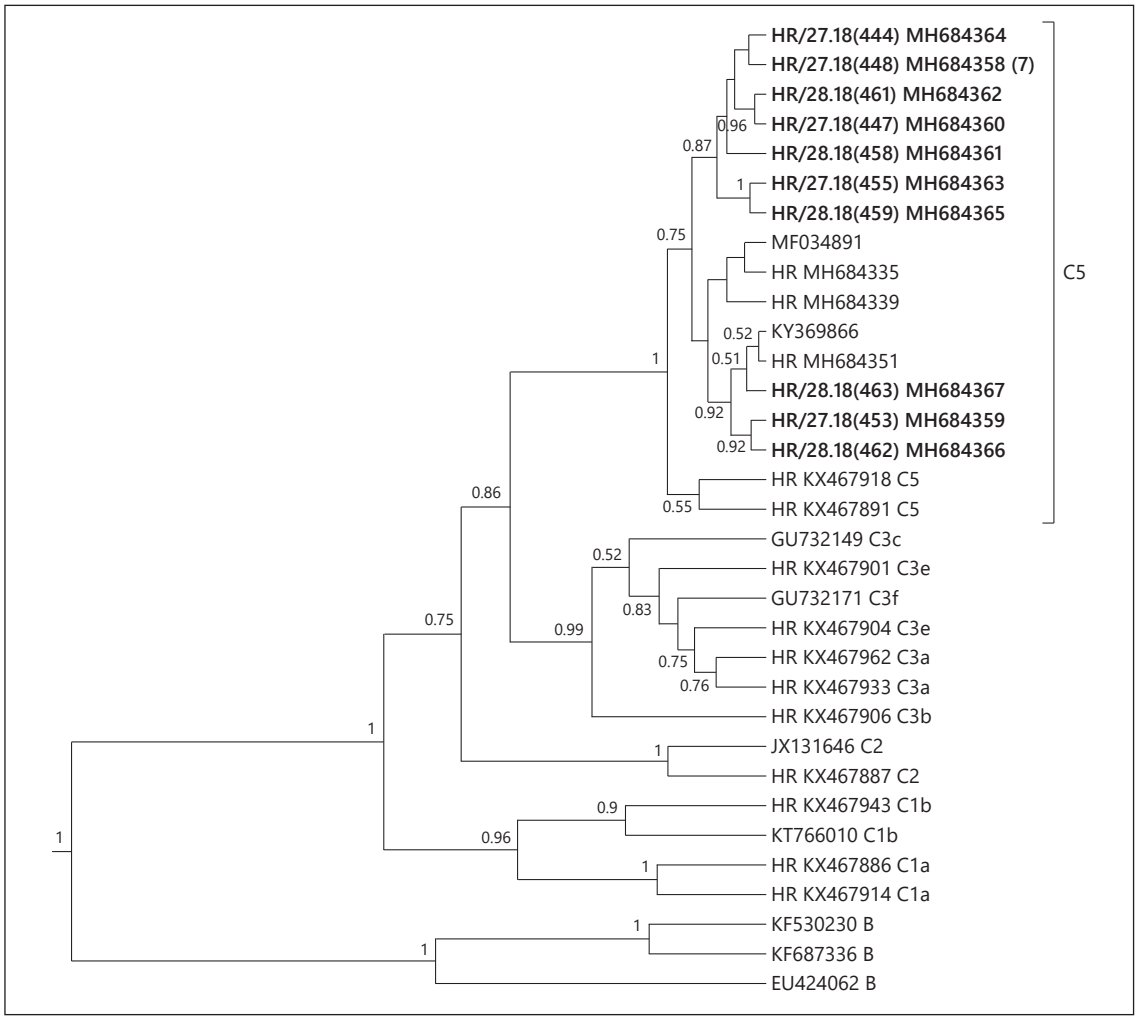

Fig. 2. Maximum clade credibility tree of HPIV3 strains based on partial HN gene (581 nt) sequences. The tree includes 10 unique sequences obtained in this study and 23 sequences retrieved from the GenBank. This phylogenetic tree was generated using the Hasegawa-Kishino-Yano substitution model with a proportion of invariable sites. Bayesian MCMC chains were run for 10 million generations, with sampling every 1,000 generations and a 1,000 burn-in to reach convergence. Only posterior probabilities above 0.5 are shown. The GenBank sequences are presented with their accession numbers, while the Croatian HPIV3 sequences from previous epidemiological studies have the prefix HR. Sequences from this study are shown in bold and are indicated with their strain identification number, followed by the accession number. The numbers of identical sequences are shown in parentheses, and the genetic classification is shown next to the strain accession number. Strains isolated from cases detected on Unit 5a (HR/28.18(463) MH684367, HR/27.18(453) MH684359, and HR/28.18(462) MH684366) are located on a separate branch.

strains differed among each other by 1–7 nucleotides, with the most differences between strains

- HR/27.18(459) MH684365 and HR/27.18(462) MH684366,
- HR/27.18(458) MH684361 and HR/27.18(462) MH684366,
- HR/27.18(459) MH684365 and HR/27.18(463) MH684367, and
- HR/27.18(453) MH684359 and HR/27.18(459) MH684365.

All strains were classified within the subcluster C5 but grouped on 2 separate branches of the phylogenetic tree (Fig. 2).

Discussion

HPIVs are frequent causative agents of respiratory infections in children, while their significance in adults, including their burden of morbidity and mortality, is much less under-

stood [4, 24]. HPIV3 is an important causative agent of ARI that can be complicated with pneumonia, especially in immunocompromised and elderly individuals. Outbreaks caused by HPIV3 have been most frequently described in neonatal units [11–13] and inpatient units with hematology/oncology patients [14–16] but also among elderly individuals residing in long-term care facilities [17–19].

This is the first study to describe an outbreak of HPIV3 among adults at a residential care facility for the disabled. This small outbreak occurred in late June, the beginning of summer. In countries with temperate climes, HPIV3 has been described to occur epidemically but most frequently in the spring (April-June) [5, 8]. Based on the 5-year retrospective study in Croatia (2011–2015), 2 epidemic peaks per year were noticed in some years (2012, 2014, and 2015), while in 2011 and 2013, only 1 epidemic peak occurred, in spring and autumn, respectively [8].

This study showed that multiplex PCR contributes to the comprehensive and rapid etiological diagnosis of ARI, timely identification of the epidemic etiology, and, consequently, the rapid introduction of measures for the prevention and control of the spread of infection in residential care facilities. Rapid diagnostics could facilitate decision-making concerning the avoidance of antimicrobial therapy where there is no demonstrable benefit or at least earlier exclusion [18]. It could also be useful for the implementation of measures for the control and prevention of nosocomial infections, such as increased surveillance and epidemic control [25].

Symptomatic and supportive therapies form the basis for the treatment of noninfluenza viral respiratory infections because effective antiviral drugs and vaccines are still not available. However, rapid molecular tests, such as multiplex PCR, can aid in the early detection of illnesses caused by these viruses and reduce the unnecessary use of antimicrobial drugs.

In our outbreak setting, all residents were mobile, and some of them were placed on the same ward. The latter means that there was ample opportunity for interaction among residents; therefore, the infection could spread when the residents were using joint facilities. This spread could have been reinforced further by someone from the personnel (i.e., nurse, caretaker, cleaning person, or social worker).

Methods of epidemic control were immediately instituted, most notably enhanced hand hygiene and disinfection, mandatory masks and gloves (barrier nursing), room ventilation, and patient placement into separate rooms. However, due to institutional restraints and lack of isolation rooms, contact isolation could not be completely implemented.

Furthermore, all visits to the residential care facility were immediately prohibited, residents were not allowed to return home, while an epidemiologist on call and sanitary engineer were notified about the outbreak. A chart was developed to determine the patient zero and subsequent spread of infection across wards, which aided in limiting the outbreak but not in pinpointing the exact source.

All the patients hospitalized with pneumonia in this study received antimicrobial therapy because they were initially thought to have bacterial pneumonia. Multiple nodular infiltrates in patients with HPIV3 pneumonia for whom other causative factors of pneumonia have been excluded have been described in the literature, particularly in the immunocompromised [26]. However, these patients still received empirical therapy with antibiotics because bacterial infection could not be excluded with certainty [26]. It is likely that our patients had severe viral pneumonia caused by HPIV3, for which antimicrobial drugs were not justified.

At this time, no antiviral drug has been approved for the treatment of HPIV infections [3]. Several novel drugs, including DAS181, seem promising in the treatment of severe disease in immunocompromised patients [27], and vaccines to decrease the burden of disease in young children are in development [28, 29]. Currently available antiviral therapy seems to be inadequate in reducing viral shedding or mortality once pneumonia has been diagnosed [30].

Thus far, residential care facilities have been rarely implicated in HPIV3 outbreaks. Conversely, hospitalization in an oncology department was associated with an increased likelihood of HPIV3 infection (aOR 2.29, 95% CI 1.78–2.96) as well as hospitalization in an organ transplantation department (aOR 3.65, 95% CI 2.80–4.76) [31]. The predominant lineages were C3c (62.3%) and C1b (24.6%), followed by sub-lineages C5 (8.7%) and C3b (2.9%) [31]. Moreover, previous studies have demonstrated that detailed molecular investigations are beneficial in identifying the transmission routes in HPIV3 outbreaks [14, 16].

Based on partial HN gene sequences, Croatian HPIV3 strains have been classified as the subcluster C5, which is in accordance with a previous epidemiological study of HPIV3 in Croatia [8] as well as other studies showing cluster C predominance [32–34]. BLAST search revealed that these strains were highly similar to strains isolated in the USA in 2016, namely KY369866 and MF034891 (Fig. 2), demonstrating that similar strains can be isolated at different times and from distant geographical sites.

Phylogenetic analysis also showed that the HPIV3 strains implicated in this outbreak are grouped on 2 sepa-

rate branches of the phylogenetic tree, albeit they all belong to HPIV3 cluster C/subcluster C5 (Fig. 2). This difference is also observed clinically, as the 2 groups of patients from whom the samples were collected were located in different rehabilitation facility units (Table 1), which means that there could have been 2 separate sources of HPIV infection.

Certain parallels can be drawn between this community-based outbreak and some reports of hospital-based outbreaks on hematology/oncology wards described in the literature [35, 36]. A research group from the UK recently described a point source outbreak of HPIV3 on an oncology pediatric unit in one major teaching hospital [35]. Akin to our report, there were no fatalities, and the main clinical impact was increased hospital stay (comparable to residents in our study that necessitated hospitalization).

However, on the hematology unit from another UK teaching hospital (with patients older than 17 years), 8 patients with PIV3 mono-infection (out of 19) died [36]. Such a significant mortality rate – despite swift introduction of manifold infection control measures – shows how PIV3 infections in adults hospitalized in posttransplant hematological units and presenting with severe immunosuppression are particularly hard to manage [36].

In addition to measures instituted in our report, these literature examples show how infection control measures during the outbreaks on hematology and pediatric oncology wards included viral screening of nasopharyngeal aspirates twice every week, dividing nurses into strictly separate cohorts and even closing the ward completely [35, 36]. There is even a consideration to include a policy for staff screening [35]; such measures are thus far not considered in Croatian conditions.

Discussed hospital cases reveal how transmission models often fail to identify transmission patterns between all the patients involved in the outbreak [35], hence it is not surprising that developing such a model in our example would prove very cumbersome. Conversely, a recent PIV3 outbreak report among US hematologic oncology patients clearly implicated visitors, unit activities, and fomites as chief sources of infection [37]. However, despite epidemiological intricacies, the clinical impact in our case is rather clear, as the illness was so severe that it necessitated a hospital stay for one-quarter of the infected individuals. Furthermore, it is frequently emphasized how molecular investigations are indispensable for outbreaks like these [35–37].

Our study has several limitations. First of all, the environmental sampling was not pursued, which could have proven pivotal in pinpointing the exact source during the outbreak. Furthermore, no air sampling was conducted in our study, although some studies have noted the potential significance of aerosols in HPIV transmission [38]. Finally, the HN gene sequences were partial and we did not conduct F gene analysis or sequence alignments for HPIV3 amplicons, as in other similar studies [14].

In conclusion, our study has shown that this health care-associated outbreak of HPIV3 infection could have been linked to multiple importation events, which subsequently resulted in branched nosocomial transmission across several units of a single rehabilitation facility. The findings strengthen the notion that routine hand washing, diligent environmental cleaning, patient isolation, and limited visitations still remain the cornerstones of preventive measures in curbing such outbreak events.

Statement of Ethics

The planning conduct and reporting of studies was in line with the Declaration of Helsinki, as revised in 2013. The research was performed in accordance with relevant guidelines/regulations and informed consent was obtained from all participants or their legal guardians. The study was approved by the Ethics Committee of the Dr. Andrija Stampar Teaching Institute of Public Health and conducted as part of the Croatian Science Foundation project entitled "New and neglected respiratory viruses in vulnerable groups of patients."

Disclosure Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Design of research: R.C. and S.L.-S. Data acquisition: R.C., E.H., N.T. Data analysis: R.C., T.K.-G., A.S., T.M., N.T., and S.L.-S. Data interpretation and writing of the manuscript: R.C., T.M., E.H., J.V., and S.L.-S. Preparation of figures: T.K.-G., A.S., and S.L.-S. All authors reviewed and approved the final version of the manuscript.

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