Received: 19 November 2021 | Accepted: 7 April 2022

DOI: 10.1002/jmv.27767

RESEARCH ARTICLE

JOURNAL OF MEDICAL VIROLOGY WILEY

Epidemiology and clinical manifestations of different enterovirus and rhinovirus types show that EV-D68 may still have an impact on severity of respiratory infections

Simon B. Larsson[1,2] | Diana Vracar[1,3] | Marie Karlsson[3] | Johan Ringlander[1,3] | Heléne Norder[1]

[1]Department of Infectious Diseases, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

[2]Department of Addiction and Dependency, Region Västra Götaland, Sahlgrenska University Hospital, Gothenburg, Sweden

[3]Department of Clinical Microbiology, Region Västra Götaland, Sahlgrenska University Hospital, Gothenburg, Sweden

Correspondence
Simon B. Larsson, Department of Infectious Diseases, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden.
Email: simon.b.larsson@gu.se

Abstract

Respiratory infections are often caused by enteroviruses (EVs). The aim of this study was to identify whether certain types of EV were more likely to cause severe illness in 2016, when an increasing spread of upper respiratory infections was observed in Gothenburg, Sweden. The EV strain in 137 of 1341 nasopharyngeal samples reactive for EV by polymerase chain reaction could be typed by sequencing the viral 5′-untranslated region and VP1 regions. Phylogenetic trees were constructed. Patient records were reviewed. Hospital care was needed for 46 of 74 patients with available medical records. The majority of the patients (83) were infected with the rhinovirus (RV). The remaining 54 were infected with EV A, B, C, and D strains of 13 different types, with EV-D68 and CV-A10 being the most common (17 vs. 14). Significantly more patients with EV-D68 presented with dyspnea, both when compared with other EV types ($p = 0.003$) and compared to all other EV and RV infections ($p = 0.04$). Phylogenetic analysis of the sequences revealed the spread of both Asian and European CV-A10 strains and 12 different RV C types. This study showed an abundance of different EV types spreading during a year with increased upper respiratory increased infections. EV-D68 infections were associated with more severe disease manifestation. Other EV and RV types were more evenly distributed between hospitalized and nonhospitalized patients. The EV type CV-A10 was also found in infected patients, which warrants further studies and surveillance, as this pathogen could cause more severe disease and outbreaks of hand, foot, and mouth disease.

KEYWORDS
CV-A10, enterovirus, epidemiology, phylogeny, rhinovirus

1 | INTRODUCTION

The *Enterovirus* (*EV*) genus of the family *Picornaviridae* has 15 classified species, EV A–L and *Rhinovirus* A–C (RV A–C). These viruses are nonenveloped, single-stranded RNA-viruses with a genome of approximately 7500 bp.[1] They infect several different mammals; EV A–D and RV A–C infect humans. Each species consists of up to 70 different types, which do not cause cross-immunity in the host. Most infections are mild with fever and/or common cold symptoms, but some types, especially those belonging to EV A–D, may cause outbreaks and manifest with meningitis, encephalitis, paralysis, neonatal sepsis, myalgia, myocarditis, or exanthema.[2–6] Notably, the three different polioviruses (PV1–3) are members of the EV C species.

For the last 10–15 years increased use of multiplex real-time polymerase chain reaction (PCR) panels has generated a large amount of data on respiratory viruses. Patients hitherto diagnosed with viral respiratory disease mainly based on clinical symptoms can now be diagnosed with the viral agent causing the disease in up to 50% of the cases.[7–9] These new diagnostic possibilities have identified new or old members belonging to EV A–D or RV A–C like viruses that engender the common cold. In addition, sequencing of partial genomes of the virus in samples from large patient cohorts with various diseases has resulted in increased knowledge of the clinical impact of different EV types.[10–12] Thus, EV-D68, which was previously considered a virus causing mild upper respiratory disease, has recently been shown to cause large outbreaks of more severe disease with complications such as acute flaccid myelitis.[4,13–15] Likewise, members belonging to species RV C have been shown to cause more severe upper and lower respiratory diseases than those belonging to RV types A or B.[16]

However, there are conflicting results regarding the pathogenicity of different types of EVs. Most studies are from hospital settings with a high risk of bias from outbreaks of meningitis and from more severely ill patients. Less is known about the prevalence of different EV types in outpatient settings and among asymptomatic patients. Also, most studies have focused on RV A–C infections and include only children.

The aim of this study was to assess epidemiological and clinical manifestations associated with certain types of EVs, and if there were differences in virus types dependent on age or disease severity in patients who sought medical care for respiratory disease.

2 | PATIENTS AND METHODS

2.1 | Study setting

Since 2006 the Virology laboratory of the Department of Clinical Microbiology (CM), Sahlgrenska University Hospital, Gothenburg, Sweden, has analyzed respiratory samples using a multiplex panel for respiratory viruses and bacteria.[17] Samples were referred from all levels in the health care system, including primary health care, emergency departments, and hospital wards. The catchment area was primarily the city of Gothenburg, the second largest city in Sweden with one million inhabitants including the surrounding area. The Virology laboratory also received samples from the surrounding region of Västra Götaland with a population of 1.7 million, including Gothenburg.

During the fall of 2016, there was a general increase in the number of samples for diagnosis of respiratory viruses sent to the Virology laboratory of the CM. To learn more about the impact of different EV and RV types on these infections, a retrospective study was initiated. The regional ethics committee of Gothenburg approved the study (no. 1078-16) and informed consent was waived for the retrospective collection of data.

2.2 | Study inclusion criteria

Nasopharyngeal and/or throat swab specimens collected at outpatient clinics, emergency departments, or hospital wards were analyzed at the CM as part of routine diagnostics. All samples included in this study had been sent to the CM from patients with respiratory disease and reactive in PCR for EV and RV, or both, during the study period, September through November 2016. Before typing, samples positive for other agents causing respiratory infections were excluded.

Patients were assigned to one of two groups: hospitalized (length of stay in hospital >1 day) and nonhospitalized (sampled in primary care or in hospital but discharged within 24 h). It should be noted that all patients had severe enough symptoms to seek medical care at the time of sampling.

2.3 | Laboratory testing

The specimens had been collected using swabs and were immediately placed in a sterile container with 1 ml of sodium chloride solution and sent to the CM either the same day or stored at +4°C until transport. At CM, specimens were analyzed directly upon arrival at the laboratory.

Total nucleic acid, including RNA, was extracted from all samples using MagnaPure Total Nucleic Acid kit (Roche, Basel, Switzerland).

In the routine analysis, a multiplex-panel real-time quantitative PCR (qPCR) system was used, an updated version of the one described in reference [17]. The panel included 16 viruses (*influenzavirus A*, *influenzavirus B*, *human respirovirus 1* and *3*, *human rubulavirus 2*, *human metapneumovirus*, *respiratory syncytial virus*, EV, *adenovirus*, human coronaviruses [229E, OC43, NL63, HKU1], and *bocavirus*) and three bacteria (*Mycoplasma pneumoniae*, *Bordertella* species, and *Chlamydia pneumoniae*).

Two PCR systems were used for the identification of EVs. One system was designed to identify most types belonging to EV A–D and the other to identify RV A. Primers and probes are described in Table S1. Due to the high sequence similarity

between some EV A–D and RV A–C types, these viruses are difficult to distinguish by qPCR. Members belonging to EV-A–D may thus react with the system designed for RV A–C and vice versa. The two systems together are, however, specific for all types of EVs.

2.4 | Sequencing

Of all 1341 patient samples reactive for EV and/or RV during 2016 (Table 1), 262 had Ct values below 30 in the routine qPCR for either EV A–D or RV A–C, and were thus selected for further analysis. A Ct value of 30 was chosen as a cut-off as previous experience of sequencing these viruses showed that with a higher Ct value, the resulting sequences are of poor quality. Extracted RNA from these 262 samples was converted to complementary DNA (cDNA) by the High Capacity cDNA Reverse Transcription kit (Applied Biosystems). The cDNA was subsequently PCR amplified with primers in both 5′-untranslated region (5′-UTR) and partial VP1, and the amplified fragments were Sanger sequenced as previously described.[18,19]

2.5 | Phylogenetic analysis of the sequences

Phylogenetic trees were built for the most frequent types, CV-A10 and EV-D68, based on VP1 sequences and RV C based on 5′-UTR sequences according to BLAST results. The sequences obtained were aligned with several other EV genomes obtained from GenBank: 60 VP1 regions of CV-A10 genomes, 74 VP1 regions of EV-D68 genomes, and 71 5′-UTR regions of RV C strains. Evolutionary distances were calculated using the Hasegawa–Kishino–Yano (HKY) algorithm with the DNADIST program in the PHYLIP package version 3.65 (58 with transition/transversion ratio of 2.41 for CV-A10, 2.62 for EV-D68, and 1.71 for RV C, and gamma correction with $\alpha = 0.88$ for CV-A10, 0.81 for EV-D68, and 0.82 for RV C. Phylogenetic trees were constructed using the unweighted pair-group method using arithmetic averages and the neighbor-joining method in the NEIGHBOR program of the PHYLIP package. The trees were visualized with the program TreeView, version 1.6.6.

2.6 | Data collection

Electronic medical records of patients whose samples had been sequenced were reviewed. Records were available for patients sampled in either the emergency department or at a hospital ward in one of the hospitals in the Region Västra Götaland. Unfortunately. We did not have access to the full medical records for patients in primary care or private clinics.

The following data were extracted: age, sex, date of onset of symptoms, date of sampling, date of hospitalization, length of stay, symptoms (fever, headache, cough, sore throat, otitis, myalgia, hoarseness, wheezing, dyspnea, tonsillar exudate, loss of appetite), saturation, need of inhalation, supplemental oxygen, intensive care, asthma development, lumbal puncture, diagnosis at discharge, underlying respiratory or other condition, and prescription of antibiotics. Due to the retrospective nature of this study, we had to rely on clinical information documented by many different physicians who treated the patients. As a consequence, all of the above-mentioned data extracted were not available for each patient.

2.7 | Statistics

Numeric variables were compared using the Mann–Whitney *U* test for comparisons between groups where variables where not normally distributed. Categorical variables were compared using the χ^2 or Fisher's exact test, where appropriate. A two-tailed $p < 0.05$ was considered statistically significant. Statistical analyses were performed in JMP 15 (SAS Institute).

3 | RESULTS

During 2016, 10 907 samples were sent to CM for analysis of respiratory viruses, an increase of 43% compared to 2014 (Table 1). Two distinct peaks with higher referral of samples were noticed for all years preceding 2016: one at the beginning of the year and one at the end (Figure 1). The second peak was more pronounced in 2016, as compared with previous years. One or several infecting agents could be identified in 47% of these samples (Table 1). EV was found with PCR in 1347 samples, a significant increase compared to

TABLE 1 Sample characteristics

Samples	2014	2015	2016
Total no. of samples analyzed for respiratory viruses	6214	10 025	10 907
Number of samples reactive for EVs including RVs	1038 (16.7%)	1154 (11.5%)	1341 (12.3%)
No. of samples reactive only in the EV-A–D system	46 (4.4%)	30 (2.6%)	106 (7.9%)
No. of samples reactive only in the RV A–C system	823 (79.3%)	1030 (89.3%)	1070 (79.8%)
No. of samples reactive in both the EV A–D and RV A–C systems	169 (16.3%)	94 (8.1%)	165 (12.3%)

Abbreviations: EVs, enteroviruses; RV, rhinovirus.

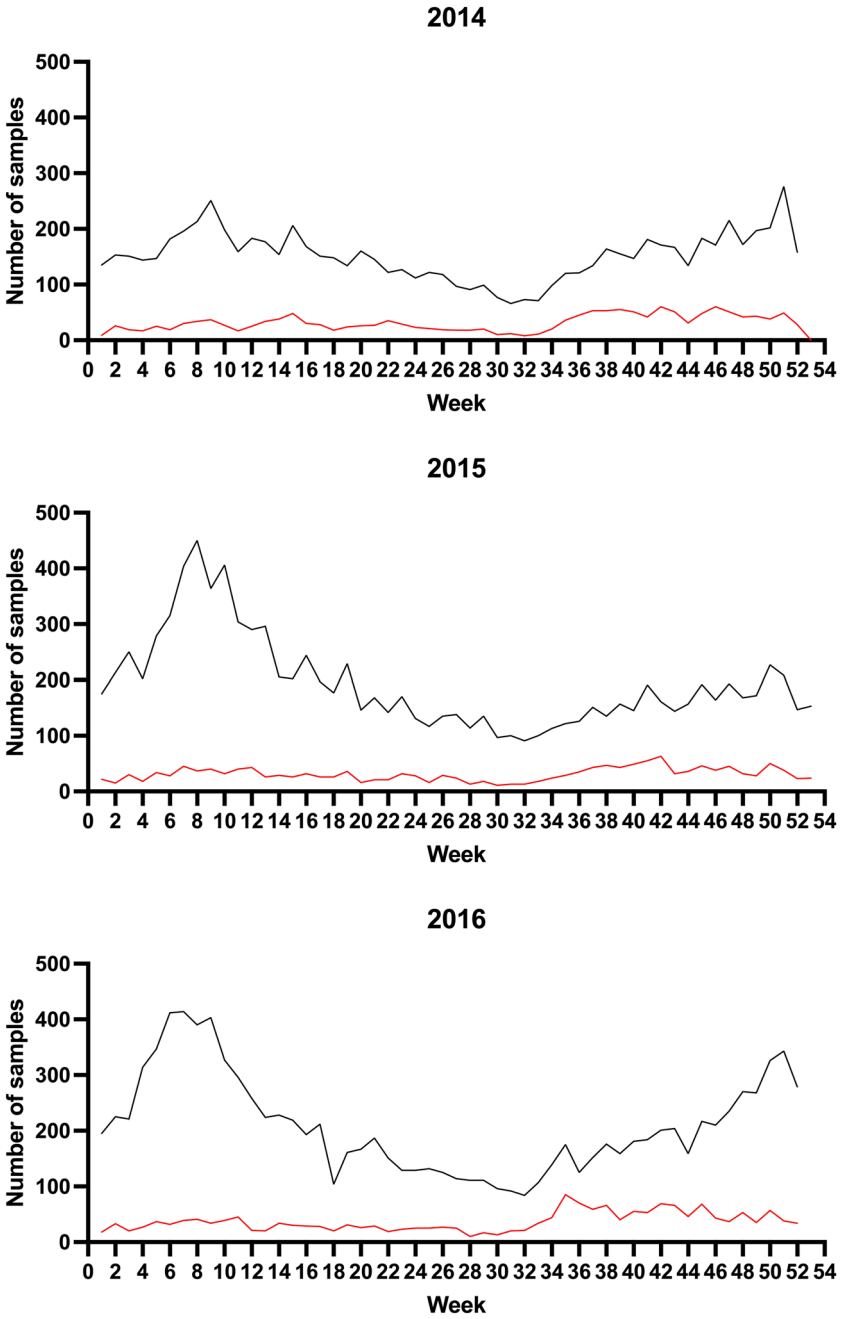

FIGURE 1 Seasonal variation of number of respiratory samples analyzed at the laboratory of Clinical Microbiology during 2014, 2015, and 2016. All respiratory samples analyzed (black line) and all enteroviruses detected (red line).

previous years ($p < 0.0001$). EV reactive samples were more common during the autumn and peaked in weeks 34–36 (Figure 2). A flowchart of sample inclusion is presented in Figure 3.

3.1 | Sequencing and phylogenetic analysis

The viral load for EV was low (Ct > 30) in 1085 out of 1347 (80%) EV reactive samples, which made them unsuitable for sequencing. For the remaining 262 samples PCR amplification and subsequent sequencing was performed. The virus type could be determined by sequencing in 137 out of 262 samples (52%; Table 2). For the remaining samples, the amplification product was either too weak for Sanger sequencing or there were multiple amplified products, making the sequences unreadable.

The type of the sequenced strains determined by BLAST and phylogenetic analysis revealed that 83 (60%) of the patients were infected by *RVs*, and of those 59% were infected with RV A strains and 14% with RV C strains (Table 2). The species could be determined for all RV strains; however, the type could not be determined for all, since most RV strains could only be amplified in the 5′-UTR-VP4 region, which has high homology between several types (Table 2). Among the other 54 patients, 45% were infected with strains belonging to EV A, mainly CV-A10, and 37% were infected with EV-D68, the only EV D type isolated. One patient was infected with EV-C109, previously known to cause upper respiratory infections.[20]

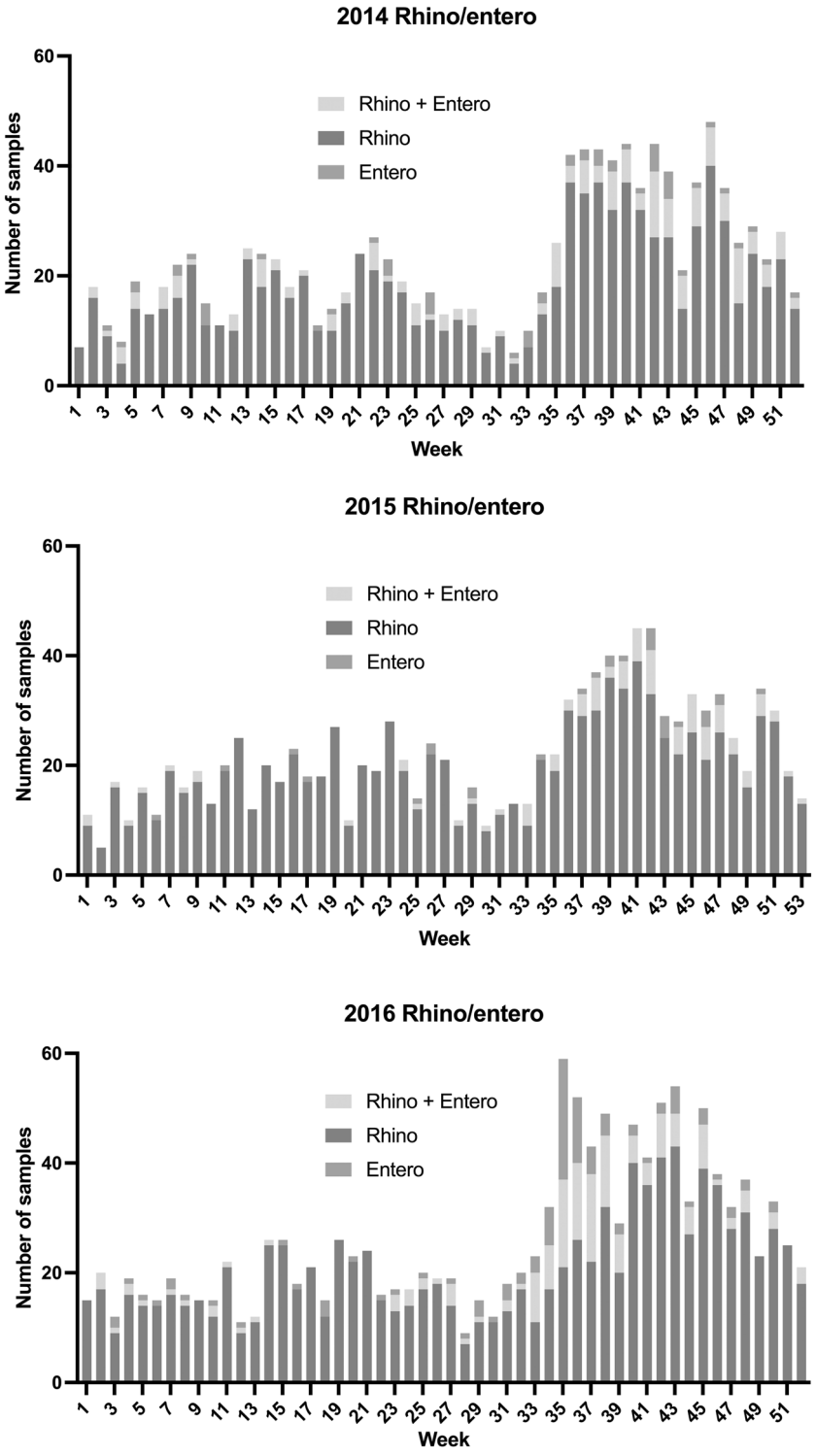

FIGURE 2 Seasonal variation of detection of enteroviruses at the laboratory of Clinical Microbiology during 2014, 2015, and 2016. Results separated according to virus type: rhinovirus (dark gray), enterovirus (medium gray), and rhinovirus/enterovirus (light gray).

3.2 | Phylogeny of sequenced samples

Phylogenetic trees of 5′-UTR of 71 RV C strains, and partial VP1 of 60 CV-A10 strains and 74 EV-D68 strains are shown in Figure 4A–C. The sequenced RV C strains in this study were found intermixed with strains from all continents on seven different main branches in the phylogenetic tree. There was thus no indication of outbreak of one specific strain (Figure 4A).

The sequences of CV-A10 separated in two different main clades. One was formed by genogroup D strains mainly from Europe and the United States, while the other main clade was formed by genogroups B/F, C, G, and E, with strains originating mainly from Asia and Australia. Most of the CV-A10 strains in GenBank originated from cases with hand foot and mouth disease (HFMD) or paralysis. Seven of the sequenced strains in this study were of genogroup D, as most European strains. Two of these strains were identical and

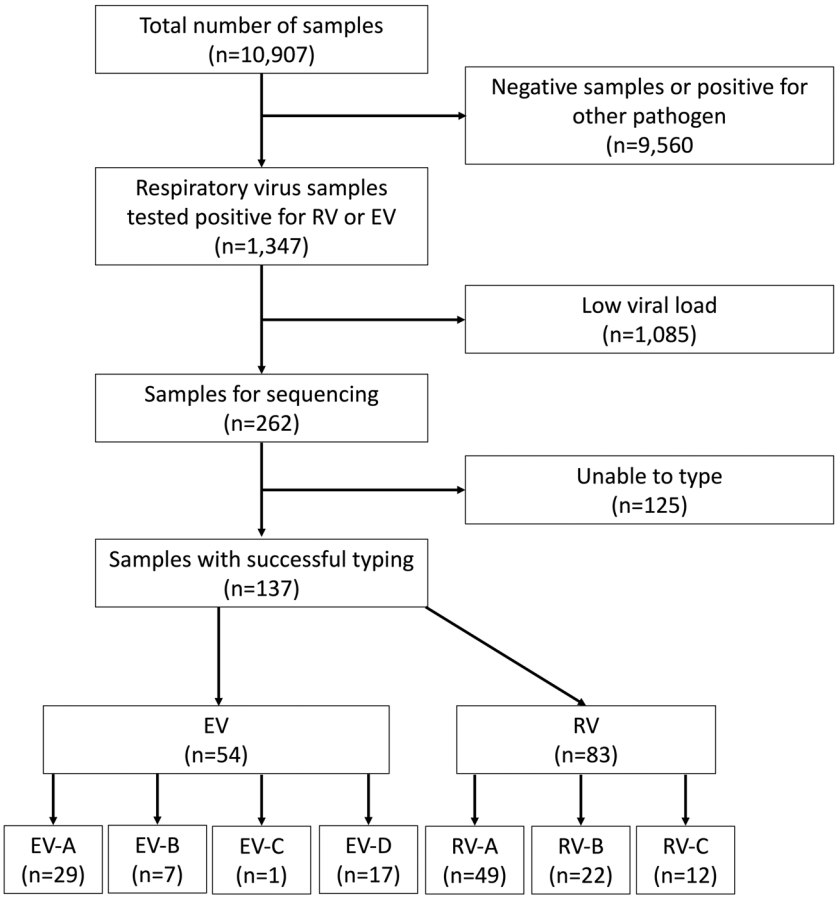

FIGURE 3 Flowchart of sample inclusion in this study. RV, rhinovirus; EV, enterovirus.

TABLE 2 Distribution EV type

Virus type	No. of reactive samples	Most common types				
EV A	31	CV-A10 (14)	CV-A6 (9)	CV-A16 (4)		
EV B	10	CVB4 (2)	E7 (2)			
EV C	2	EV-C109 (2)				
EV D	25	EV-D68 (25)				
RV A	64	Untypeable (8)[a,b]	HRV-A46 (4)	HRV-A46 (3)	HRV-A51 (3)	HRV-A62 (3)[b]
RV B	25	HRV-B6 (6)	HRV-B72 (6)	HRV-B42 (3)	HRV-B27 (2)	Untypeable[a] (2)
RV C	38	Untypeable[a] (17)	HRV-C49 (4)	HRV-C6 (3)	HRV-C15 (3)	HRV-C43 (3)

Abbreviations: EVs, enteroviruses; RV, rhinovirus; UTR, untranslated region.

[a]The type could not be determined for all sequences based only on the 5′-UTR/VP4 region.

[b]Three strains each were also typed as HRV-A15 and HRV-A21.

another strain diverged by only two nucleotides from these two strains, indicating a common source of infection. There were in addition three strains forming separate clades and may represent new European genogroups. Three of the other CV-A10 strains were similar to Chinese genogroup E strains causing HFMD. One additional strain sequenced in this study formed a separate branch with some similarities to the genogroup E strains (Figure 4B).

The sequenced EV-D68 strains were intermixed with other EV-D68 strains, isolated the same year, in the phylogenetic tree (Figure 4C). The strains diverged from each other and there was no indication of a common source of infection. Only one strain was identical to two Swedish strains isolated in 2016; however, this strain had also been isolated in Japan the year before.

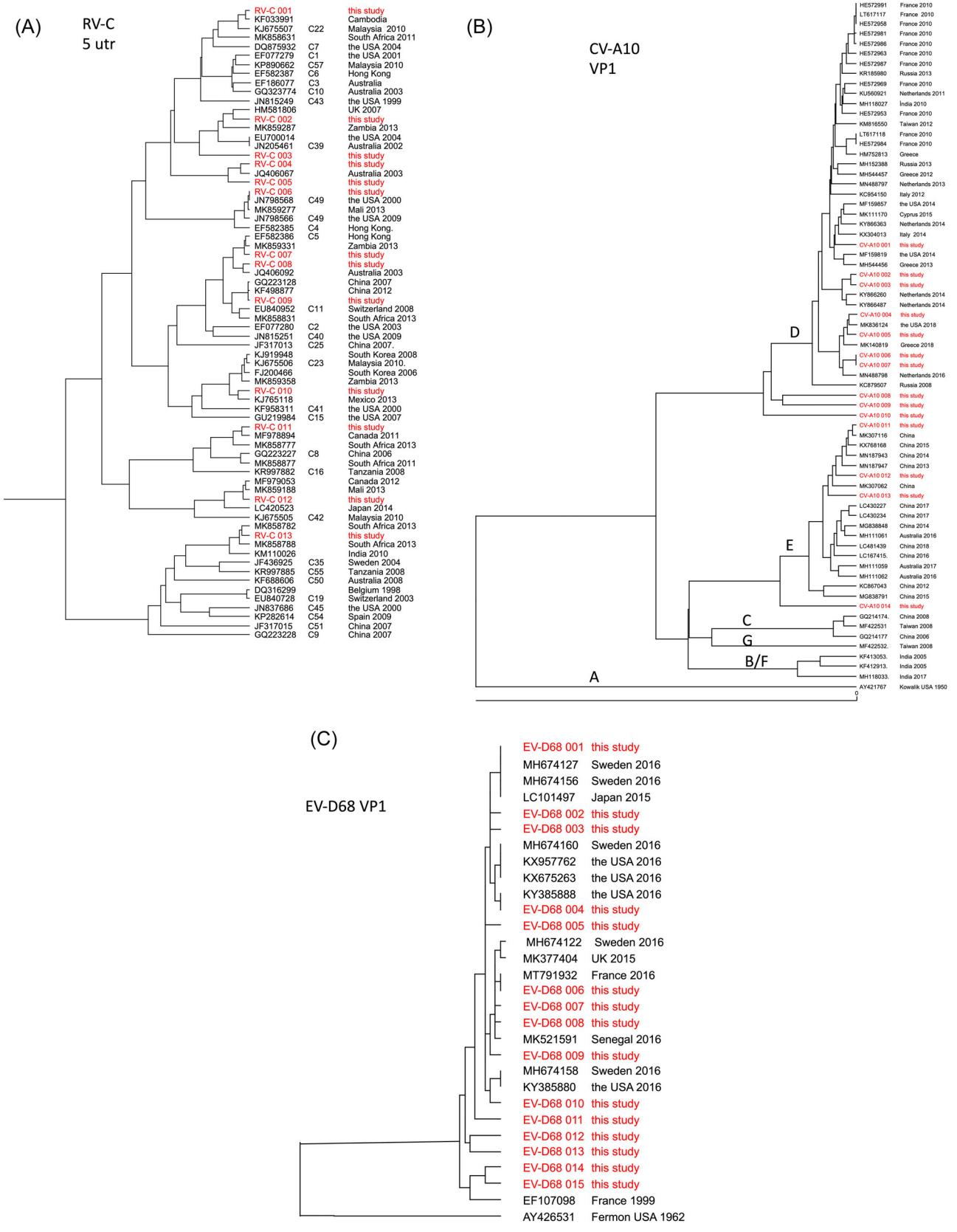

FIGURE 4 Phylogenetic trees of sequenced samples. (A) Rhinovirus C, (B) coxsackievirus A10, and (C) enterovirus D68.

TABLE 3 Patient characteristics

Categories	All	Hospitalized	Nonhospitalized	
Age (years)	5.09 (0.02–89.1)	15.3 (0.02–89.1)	3.4 (0.07–76.8)	$p = 0.007$[a]
Sex (M/F)	88/48	27/19	62/29	n.s.[b]
Virus type (EV/RV)	54/83	16/30	38/53	n.s.[b]
RV C versus RV A–B	12/70	3/27	9/43	n.s.[b]
EV-D68 versus EV A–C	17/37	10/6	7/31	$p = 0.003$[b]

Note: Age is represented as median (range).

Abbreviations: EVs, enteroviruses; n.s., not significant; RV, rhinovirus.

[a]Wilcoxon's rank-sum test.

[b]Fisher's exact test.

3.3 | EV and RV types in relation to clinical data

The prevalence of infecting virus species is presented in Table 3 along with demographic data for the patients. The patients were divided into two groups, based on whether they were hospitalized >1 day ($n = 46$) or not ($n = 91$). Five patients had been sampled outside the Region Västra Götaland and all of them were hospitalized for more than one day.

Hospitalized patients were significantly older than those who were not hospitalized (median age 15.3 vs. 3.4 years, $p = 0.007$). There were more males than females infected, 88 versus 49, and the age distribution revealed more EV A–D infected in patients younger than 15 years (40 vs. 4), while the RV infected could be found in all age groups (Table 3).

In hospitalized patients, EV-D68 was more common than the other EV types as compared with nonhospitalized patients ($p = 0.003$; Table 3). For other EV or RV types, there was no difference in distribution between the two groups of patients. When results from children <5 years of age were analyzed separately, results did not differ from analysis including all ages.

3.4 | Clinical manifestations and virus type

Clinical data were available for 74 patients who had sought care at a hospital or emergency ward. Forty-six patients were hospitalized more than one day and the remaining 38 patients were discharged within 24 h. Due to lack of consistency in documentation in medical records, only clinical signs or symptoms documented in at least 80% of patients were discussed below.

3.5 | Dyspnea

A significantly higher prevalence of EV-D68 was found among patients with dyspnea, both when compared with patients infected with other EV types ($p = 0.005$) and compared to all other EV and RV infections ($p = 0.04$). No association was found between dyspnea and RV C infection as compared with the other RV types (Table 4).

TABLE 4 Comparison of reported dyspnea related to virus type

Virus type	Dyspnea	No dyspnea	
EV versus RV	7/15	18/36	n.s[a]
RV C versus RV A–B	0/15	6/30	n.s.[a]
EV-D68 versus EV AB + RV	7/15	6/48	$p = 0.04$[a]
EV-D68 versus EV A–B	7/0	6/12	$p = 0.005$[a]

Abbreviations: EVs, enteroviruses; n.s., not significant; RV, rhinovirus.

[a]Fisher's exact test.

3.6 | Diagnosis and other clinical parameters

Patients infected with EV-D68 were significantly more often treated with inhalation of bronchodilating substances (y/n = 7/4 vs. 0/10) than those infected with other EV types ($p = 0.004$). There were no significant differences in the distribution of infecting EV type when comparing diagnosis (upper respiratory infection vs. any other comorbidity), need for intensive care, length of stay (≤1 vs. >1 days), use of antibiotics, need for supplemental oxygen, and underlying respiratory disease or other underlying diseases.

3.7 | Patients with multiple samples

Four patients contributed with more than one sample during the study period (Table S2). In two cases, these samples were taken within 24 h and 5 days, respectively. These samples contained the same virus type. Samples taken 9 or more days apart contained different virus types, indicating a new infection or double infection with one virus type being more prevalent in the first sample and the other virus type more prevalent in the second sample.

3.8 | Hospital care within 1 month after respiratory virus sampling

Among patients sampled in primary health care ($n = 62$), six sought hospital care within 1 month and thus had a medical record. All of

these patients were diagnosed with an upper respiratory infection. Five patients were younger than 5 years of age. None of the patients was hospitalized.

3.9 | Clinical manifestations for EV type CV-A10

Fourteen patients were infected by CV-A10 strains. Eleven of those were below 5 years of age, and one was hospitalized due to another disease and diagnosed with upper respiratory infection during the hospital stay. Two patients who sought care in the emergency department were diagnosed with an upper respiratory infection.

4 | DISCUSSION

This study showed a high variability of EV and RV types circulating in the Gothenburg area in Sweden during 2016, a year with an increased number of upper respiratory infections. We did not find any obvious outbreaks of certain strains, although there was a relatively high number of CV-A10 and EV-D68 among those infected with the EV species A through D. Meanwhile, RV A strains of several different types were most common among the RV infected. The lack of a few main species or strains causing outbreaks reveals a large spread of many virus types simultaneously. Interestingly, also during times of low incidence, it was shown that patients infected with EV-D68, which previously was considered to cause only milder infections, more often had severe disease.

The new strains of EV-D68 found in this study seem to be part of strains still circulating in the society after the large global outbreak in 2014 and seem to have substituted the previously isolated strains, causing milder disease. Even if there were no significant associations with disease severity for CV-A10 and other EV types, they were prevalent among patients with respiratory illness and some of these types may develop outbreaks if the herd immunity becomes low and warrants surveillance, especially for CV-A10 and EV-D68.

Patients were divided into two groups, those hospitalized >1 day and those not hospitalized. There were small differences in EV- and RV-type distribution between patients in the two groups. However, for all patients the symptoms caused them to seek medical care, which implies they were more or less severe. EV-D68 was found to be significantly more prevalent in patients suffering from dyspnea, patients in need of bronchodilators, and patients in need of hospitalization. The latter findings were also significant in the subgroup of patients under 5 years of age, which supports that the result was not biased by age, which was found to be a risk factor for hospitalization.

Sequencing of respiratory samples positive for EVs revealed a wide range of virus types probably representing a typical distribution associated with the community spread of EV during the beginning of the season for the common cold in Sweden. Similar distribution patterns of different EVs have been shown in several previous studies.[21,22]

EV-D68 has shown seasonality and is often associated with severe disease.[23–25] A recent study by CDC suggests a biennial pattern in the United States.[26] In the present study, species EV D was more prevalent in 2016 than in the two previous years, and interestingly, EV-D68 was very much dominant also during the study period. The unusually high number of samples during 2016 might partly reflect a higher virulence of EV-D68. In addition, patients with EV-D68 more often had dyspnea and bronchodilation treatment, compared to patients infected with other EV and RV types. Our and other findings on the disease severity of EV-D68 infections highlight the importance of surveillance of this pathogen.[4,13–15]

Usually, CV-A6 and CV-A10 are found in patients presenting with HFMD.[27,28] Respiratory symptoms associated with these types have been reported in a previous study.[29] In a recent review, it was pointed out that CV-A10 has been detected more often in HFMD in the last 10–20 years[6] and a recent study from Korea showed that both CV-A6 and CV-A10 were commonly found in an EV surveillance program from 2012 to 2019.[10] In the present study, these types were also found in clinical nasopharynx samples. All cases with CV-A10 but one were found among nonhospitalized patients, which may imply that it is less likely to cause severe disease.

Several studies report an association between disease severity and virus type,[12,16,28,30,31] while other studies found that disease severity was unrelated to EV type.[32,33] Most previous studies only included hospitalized patients and therefore comparison between patients suffering from both mild and severe diseases could not unbiasedly be made. For instance, a recent study from Brazil found RV in almost a quarter of cases with severe bronchiolitis in small children, but the samples were not typed.[34] The present study includes patients from both hospitals (emergency departments, hospital wards, and intensive care units) and primary care centers.

Our results extend to those previously reported by Comte et al.,[35] who did not find any differences between EV type (RV A–C and EV-D68) in mild or severely ill patients.[35] In their study 70% of patients were defined as not having a severe infection, comparable to our results (66% nonhospitalized patients). A new study from Denmark even suggests that RV can contribute to less severe disease when codetected in common human coronavirus infection.[36]

Correct diagnosis of the specific pathogen causing respiratory symptoms could be beneficial also in mild cases and may contribute to less use of antibiotics.[37] In the present study, 15 out of 33 nonhospitalized patients, for whom a medical record was available, were prescribed antibiotics. Given the positive test results for RV or EV and no evidence of bacterial infections, antibiotic treatment could presumably have been avoided for these patients.

This study supports the paradigm that different RV and EV types do not induce protective cross-immunity in humans. In patients with two samples taken more than 8 days apart, a different EV type was identified in the second sample, suggesting that there is no clinically relevant cross-immunity between EV and RV types.[38]

An age difference between patients with mild and severe disease was noticed. As there was no difference in EV-type distribution between the two groups, this suggests that children may have a

milder disease when infected by any RV/EV type. However, we cannot rule out that older patients, in general, more often have severe underlying conditions, which may impact the clinical outcome and symptoms, as suggested by previous studies.[38,39]

Our study has some limitations. First, this study was a retrospective study and we could only gather clinical data documented in the medical records. Documentation in medical records was not consistent and data on specific signs and symptoms was lacking in many patients. For patients sampled in primary care, we did not have access to the full medical records. Most samples initially identified from the routine diagnostics had to be excluded for further analysis due to the high Ct value. Thus we do not know their EV types. The number of samples finally included in the analyses was smaller than we hoped when planning the study. As a consequence, we might have missed associations significant in a larger sample size. With these precautions, our results probably reflect the natural, broad spectrum of patients with EV and RV, and thereby provide more realistic results, such as associations between disease severity and EV/RV types, as compared to many previous studies that only included hospitalized cases.

Our findings suggest that most EV and RV types have a weak association to symptoms and severity of respiratory infections caused by the viruses, with the exception of EV-D68, which may cause more severe disease and more often dyspnea. We warrant further studies on this EV type to elucidate its prevalence among different patients and its role in the pathogenesis of severe respiratory infection. Also, the role of CV-A10 in respiratory disease merits further investigation. There is a great need for broad prospective studies on EV and RV types, including patients suffering from mild or severe illness, from both hospital and primary care.

AUTHOR CONTRIBUTIONS

Heléne Norder and Simon B. Larsson designed the study. Heléne Norder, Diana Vracar, Marie Karlsson, and Simon B. Larsson were involved in data collection. Heléne Norder and Simon B. Larsson performed the statistical analysis. Heléne Norder, Diana Vracar, Simon B. Larsson, and Johan Ringlander participated in the data analysis. Heléne Norder and Simon B. Larsson wrote the original draft of the manuscript. Diana Vracar, Marie Karlsson, and Johan Ringlander contributed to the revision of further drafts. All authors have critically read and commented on draft versions of the report and approved the final version.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author, Simon B. Larsson, upon reasonable request.

ETHICS STATEMENT

The regional ethics committee of Gothenburg approved the study (no. 1078-16) and informed consent was waived for the retrospective collection of data.

ORCID

Simon B. Larsson http://orcid.org/0000-0002-0964-6137
Johan Ringlander http://orcid.org/0000-0002-8483-6504
Heléne Norder http://orcid.org/0000-0002-7528-3872

REFERENCES

1. Lukashev AN, Vakulenko YA, Turbabina NA, Deviatkin AA, Drexler JF. Molecular epidemiology and phylogenetics of human enteroviruses: is there a forest behind the trees? *Rev Med Virol*. 2018;28:1-10. doi:10.1002/rmv.2002
2. Robinson CP, Busl KM. Neurologic manifestations of severe respiratory viral contagions. *Crit Care Explor*. 2020;2:e0107.
3. Dickson C, Ho Mi Fane B, Squires SG. Acute flaccid myelitis in Canada, 2018 to 2019. *Can Commun Dis Rep*. 2020;46:349-353.
4. McLaren N, Lopez A, Kidd S, et al. Characteristics of patients with acute flaccid myelitis, United States, 2015–2018. *Emerg Infect Dis*. 2020;26:26.
5. Chuang Y-Y, Huang Y-C. Enteroviral infection in neonates. *J Microbiol Immunol Infect*. 2019;52:851-857.
6. Bian L, Gao F, Mao Q, et al. Hand, foot, and mouth disease associated with coxsackievirus A10: more serious than it seems. *Expert Rev Anti Infect Ther*. 2019;17:233-242.
7. Nickbakhsh S, Thorburn F, Wissmann VON, et al. Extensive multiplex PCR diagnostics reveal new insights into the epidemiology of viral respiratory infections. *Epidemiol Infect*. 2016;144:2064-2076.
8. Brittain-Long R, Westin J, Olofsson S, Lindh M, Andersson L-M. Prospective evaluation of a novel multiplex real-time PCR assay for detection of fifteen respiratory pathogens—duration of symptoms significantly affects detection rate. *J Clin Virol*. 2010;47:263-267.
9. Leotte J, Trombetta H, Faggion HZ, et al. Impact and seasonality of human rhinovirus infection in hospitalized patients for two consecutive years. *J Pediatr*. 2017;93:294-300.
10. Kang HJ, Yoon Y, Lee Y-P, et al. A Different epidemiology of enterovirus a and enterovirus B co-circulating in Korea, 2012–2019. *J Pediatric Infect Dis Soc*. 2021;10:398-407.
11. Pellegrinelli L, Galli C, Primache V, et al. Emerging non-polio enteroviruses recognized in the framework of the acute flaccid paralyses (AFP) surveillance system in Northern Italy, 2016–2018. *Int J Infect Dis*. 2021;106:36-40.
12. Molet L, Saloum K, Marque-Juillet S, et al. Enterovirus infections in hospitals of Ile de France region over 2013. *J Clin Virol*. 2016;74:37-42.
13. Yea C, Bitnun A, Branson HM, et al. Association of outcomes in acute flaccid myelitis with identification of enterovirus at presentation: a Canadian, nationwide, longitudinal study. *Lancet Child Adolesc Health*. 2020;4:828-836.
14. Antona D, Kossorotoff M, Schuffenecker I, et al. Severe paediatric conditions linked with EV-A71 and EV-D68, France, May to October 2016. *Eurosurveillance*. 2016;21:1-4. doi:10.2807/1560-7917.ES.2016.21.46.30402
15. Du J, Zheng B, Zheng W, et al. Analysis of enterovirus 68 strains from the 2014 North American outbreak reveals a new clade, indicating viral evolution. *PLoS One*. 2015;10:e0144208.
16. Erkkola R, Turunen R, Räisänen K, et al. Rhinovirus C is associated with severe wheezing and febrile respiratory illness in young children. *Pediatr Infect Dis J*. 2020;39:283-286.

17. Brittain-Long R, Nord S, Olofsson S, Westin J, Anderson L-M, Lindh M. Multiplex real-time PCR for detection of respiratory tract infections. *J Clin Virol*. 2008;41:53-56.
18. Ayukekbong JA, Fobisong C, Lindh M, Nkuo-Akenji T, Bergström T, Norder H. Molecular analysis of enterovirus in cameroon by partial 5′UTR-VP4 gene sequencing reveals a high genetic diversity and frequency of infections. *J Med Virol*. 2014; 86:2092-2101.
19. Norder H, Bjerregaard L, Magnius L, Lina B, Aymard M, Chomel J-J. Sequencing of "untypable" enteroviruses reveals two new types, EV-77 and EV-78, within human enterovirus type B and substitutions in the BC loop of the VP1 protein for known types. *J Gen Virol*. 2003; 84:827-836.
20. Ng TFF, Yglesias JA, Stevenson-Yuen TA, et al. Characterization of the genome sequences of enterovirus C109 from two respiratory disease cases in Florida, 2016. *Microbiol Resour Announc*. 2018;7: 1-2.
21. van der Linden L, Bruning AHL, Thomas XV, et al. A molecular epidemiological perspective of rhinovirus types circulating in Amsterdam from 2007 to 2012. *Clin Microbiol Infect*. 2016;22: 1002.e9-1002.e14.
22. Chen W-J, Arnold JC, Fairchok MP, et al. Epidemiologic, clinical, and virologic characteristics of human rhinovirus infection among otherwise healthy children and adults. *J Clin Virol*. 2015;64: 74-82.
23. Pons-Salort M, Oberste MS, Pallansch MA, et al. The seasonality of nonpolio enteroviruses in the United States: patterns and drivers. *Proc Natl Acad Sci USA*. 2018;115:3078-3083.
24. Bragstad K, Jakobsen K, Rojahn AE, et al. High frequency of enterovirus D 68 in children hospitalised with respiratory illness in Norway, autumn 2014. *Influenza Other Respir Viruses*. 2015;9: 59-63.
25. Engelmann I, Fatoux M, Lazrek M, et al. Enterovirus D68 detection in respiratory specimens: association with severe disease. *J Med Virol*. 2017;89:1201-1207.
26. Shah MM, Perez A, Lively JY, et al. Enterovirus D68-associated acute respiratory illness—new vaccine surveillance network, United States, July–November 2018–2020. *Morb Mortal Wkly Rep*. 2021; 70:1623-1628.
27. de Sousa IP Jr, Giamberardino HI, Raboni SM, et al. Simultaneous enterovirus EV-D68 and CVA6 infections causing acute respiratory distress syndrome and hand, foot and mouth disease. *Virol J*. 2021;18:88.
28. Yang X, Li Y, Zhang C, et al. Clinical features and phylogenetic analysis of severe hand-foot-and-mouth disease caused by coxsackievirus A6. *Infect Genet Evol*. 2020;77:104054.
29. Lu Q-B, Zhang X-A, Wo Y, et al. Circulation of coxsackievirus A10 and A6 in hand-foot-mouth disease in China, 2009–2011. *PLoS One*. 7, 2012:e52073.
30. Lauinger IL, Bible JM, Halligan EP, et al. Patient characteristics and severity of human rhinovirus infections in children. *J Clin Virol*. 2013; 58:216-220.
31. Lee W-M, Lemanske RF Jr, Evans MD, et al. Human rhinovirus species and season of infection determine illness severity. *Am J Respir Crit Care Med*. 2012;186:886-891.
32. McCulloch DJ, Sears MH, Jacob JT, et al. Severity of rhinovirus infection in hospitalized adults is unrelated to genotype. *Am J Clin Path*. 2014;142:165-172.
33. Ljubin-Sternak S, Meštrović T, Ivković-Jureković I, et al. The emerging role of rhinoviruses in lower respiratory tract infections in children—clinical and molecular epidemiological study from Croatia, 2017–2019. *Front Microbiol*. 2019;10:2737.
34. Bastos JCS, Simas PVM, Caserta LC, et al. Rhinoviruses as critical agents in severe bronchiolitis in infants. *J Pediatr*. 2021: S0021755721001674.
35. Comte A, Bour J-B, Darniot M, Pitoiset C, Aho-Glélé LS, Manoha C. Epidemiological characteristics and clinical outcomes of human rhinovirus infections in a hospitalized population. Severity is independently linked to RSV coinfection and comorbidities. *J Clin Virol*. 2020;125:104290.
36. Weiss ZF, Cunha CB, Chambers AB, et al. Opportunities revealed for antimicrobial stewardship and clinical practice with implementation of a rapid respiratory multiplex assay. *J Clin Microbiol*. 2019;57:1-10.
37. Zlateva KT, de Vries JJ, Coenjaerts FE, et al. Prolonged shedding of rhinovirus and re-infection in adults with respiratory tract illness. *Eur Respir J*. 2014;44:169-177.
38. Greenberg SB. Viral respiratory infections in elderly patients and patients with chronic obstructive pulmonary disease. *Am J Med*. 2002;112(suppl 6A):28S-32S.
39. Martin EK, Kuypers J, Chu HY, et al. Molecular epidemiology of human rhinovirus infections in the pediatric emergency department. *J Clin Virol*. 2015;62:25-31.
40. Heimdal I, Valand J, Krokstad S, Moe N, et al. Hospitalized Children With Common Human Coronavirus Clinical Impact of Codetected Respiratory Syncytial Virus and Rhinovirus. *Pediatr. Infect. Dis. J.* 2022; 41(3):e95–e101. https://doi.org/10.1097/inf.0000000000003433

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Larsson SB, Vracar D, Karlsson M, Ringlander J, Norder H. Epidemiology and clinical manifestations of different enterovirus and rhinovirus types show that EV-D68 may still have an impact on severity of respiratory infections. *J Med Virol*. 2022;94:3829-3839. doi:10.1002/jmv.27767