Eur J Clin Microbiol Infect Dis (2013) 32:947–954
DOI 10.1007/s10096-013-1832-x

ARTICLE

Rhinovirus infections in western Sweden: a four-year molecular epidemiology study comparing local and globally appearing types

M. Sansone · M. Andersson · R. Brittain-Long · L.-M. Andersson · S. Olofsson · J. Westin · M. Lindh

Received: 3 October 2012 / Accepted: 24 January 2013 / Published online: 23 February 2013

Abstract Human rhinovirus (HRV) is a highly prevalent pathogen and a major cause of acute respiratory tract infection (ARTI). HRV express less seasonality than other viral ARTIs, which typically appear as seasonal epidemics lasting for 1–2 months. The aim of this study was to investigate the seasonal patterns of HRV types over four consecutive years in one geographic region. HRV identified in respiratory samples from 114 patients over a four-year period were analysed by VP4/VP2 sequencing. HRV-A was found in 64, HRV-B in 11 and HRV-C in 37 cases. Overall, 33 different HRV-A types, nine B types and 21 C types were found. As many as 21 of the HRV types appeared during several seasons, with a maximum time-span of four years. Some types appeared during successive seasons and, in some cases, phylogenetic analysis indicated extended periods of circulation locally. Most of the strains were closely related to HRV identified in other parts of the world during the same time period. HRV strains that circulate locally represent many types and seem to reflect that HRV infections are highly globalised. The existence of simultaneous or successive epidemics with different HRV types in combination with the ability of each type to remain in the local population over extended periods of time may contribute to explaining the high rate of HRV infections.

M. Sansone · M. Andersson · L.-M. Andersson · S. Olofsson · J. Westin · M. Lindh (✉)
Department of Infectious Diseases, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Guldhedsgatan 10B, 413 36 Gothenburg, Sweden
e-mail: magnus.lindh@microbio.gu.se

R. Brittain-Long
Acute Medical Assessment Unit, Aberdeen Royal Infirmary, Aberdeen, Scotland, UK

Background

Human rhinovirus (HRV) is the most prevalent viral respiratory pathogen in humans and the predominant aetiology of the "common cold". It has also been associated with influenza-like illness [1], otitis media [2] and exacerbations of asthma and chronic obstructive pulmonary disease [3, 4]. HRV infections, thus, represent a significant global health burden, as well as substantial cost for health care and lost productivity [5].

HRV belongs to the *Enterovirus* genus, family Picornaviridae, and is genetically diverse. A multitude of serotypes and genotypes (currently around 150) have been described, hereafter referred to as types. Previously being classified into two species, HRV-A and HRV-B, sequencing-based studies revealed a third species in 2006, HRV-C, which circulates worldwide [6–10]. HRV-C was initially reported to cause more severe symptoms than HRV-A and HRV-B, but further studies have reported similar clinical presentation across HRV species [9].

In temperate regions, acute respiratory tract infection (ARTI) of viral aetiology typically appear as temporally restricted epidemics during the winter season [11]. In particular, influenza A and respiratory syncytial virus (RSV) infections cause distinct epidemics lasting 1–2 months. In contrast, infections caused by HRVs appear throughout the entire year, sometimes with peaks in early autumn and spring [12–15]. The overall high incidence and observed lower degree of seasonality for HRV could reflect that there are several parallel or successive epidemics with different HRV types, each appearing in a time-limited fashion, similar to what is observed for other viruses causing ARTIs. A multitude of HRV types during each season has been observed earlier, both a long time ago by surveillance studies using viral culture [16] and more recently by molecular epidemiology investigations [17–20].

The aim of the study was to identify HRV types circulating in one geographic area across several seasons and to compare the strains detected in this area with published HRV sequences retrieved from other parts of the world.

Methods

Samples

During the period from November 2006 through September 2010, 11,498 respiratory samples were sent to the Virological Laboratory at the Sahlgrenska University Hospital for the diagnosis of ARTI by a multiple real-time polymerase chain reaction (PCR) panel. HRV was detected in 16 % of all the samples and in 25 % of samples from children below 5 years of age. The samples were sent from various health care units within a catchment area of approximately 500,000 inhabitants in the city of Gothenburg on the west coast of Sweden. In the present study, 170 samples positive for HRV (9 % of the total number of samples positive for HRV) were selected for typing by sequencing of the VP4/VP2 region. The samples were selected so as to represent the autumn as well as the spring seasons each year.

Real-time PCR

The multiple real-time PCR assay, which targets 15 respiratory agents, has been described previously [21]. One of the reaction wells contains oligonucleotides targeting rhinovirus, *Mycoplasma pneumoniae* and coronavirus 229E. Conserved parts of the rhinovirus genome are targeted with a mixture of three forward primers (GGTGTGAAGA GCCSCRTGTGCT, GGTGTGAAGACTCGCATGTGCT, GGGTGYGAAGAGYCTANTGTGCT), one reverse primer (GGACACCCAAAGTAGTYGGTYC) and one minor groove binding probe (TCCGGCCCCTGAATG).

Sequencing of the VP4/VP2 region and phylogeny

After total nucleic acid extraction in a MagNA Pure LC instrument (Roche, Branchburg, NJ, USA), amplification was performed using the primers Rhino_547F, ACCRACTACTTTGGGTGTCCGTG and Rhino_1125R, ACATRTTTTSiCCAAAiAiiCCCAT in a first PCR and Rhino_547F and Rhino_1087R, TCWGGiARYTTCCA MCACCAiCC in a second PCR (i = inosine). Cycle sequencing was carried out in both directions using ABI BigDye Terminators (Life Technologies, Carlsbad, CA, USA) and Rhino_547F and Rhino_1087R as primers, and the sequences were read in an ABI 3130XL instrument and assembled using the Lasergene software (DNASTAR, Inc., Madison, WI, USA). VP4/VP2 sequences in this study have been assigned EMBL accession numbers HE993758–HE993869.

Analysis strategy

The obtained sequences from local samples were compared with reference sequences from other geographical areas representing known HRV types. These references included 74 HRV-A, 24 HRV-B and 50 HRV-C sequences, classified as suggested by the International Committee on Taxonomy of Viruses (ICTV) Picornaviridae Study Group, http://www.picornastudygroup.com (with provisional classification for 14 HRV-C sequences). In order to retrieve the 5–10 published sequences of the same type with the closest similarity, a BLAST search was also performed for each of our sequences. These sequences were then used for detailed phylogenetic analysis of the types that were found in our population during several seasons.

Phylogeny and classification

A segment of 395 nucleotides (nt) from the analysed strains were aligned along with reference sequences, and phylogenetic trees were constructed by maximum-likelihood analysis using the MEGA5 software [22], applying pairwise deletion for missing data and the Tamura–Nei model for nucleotide substitutions. Type assignment of our sequences was based on >90 % nt similarity to a reference sequence or clustering with a reference sequence in phylogenetic analysis with a bootstrap value above 70.

Genetic distances between and within HRV-A, HRV-B and HRV-C were compared by Student's *t*-test.

Results

HRV types

In all, 114/170 samples produced amplicons and sequences of sufficient length and quality for phylogenetic comparison. By VP4/VP2 sequence analysis, we found HRV-A in 64, HRV-B in 11 and HRV-C in 37 cases. In two cases that were reactive for both HRV and enterovirus in real-time PCR, coxsackie virus A9 and enterovirus 68 were detected. There were 33 different HRV-A types, nine different B types and 21 different C types. As shown in Table 1, the most commonly detected types were C9 (n=7) and A56 (n=5).

Forty-seven percent (54/114) of the samples were obtained from children (age<18 years) and 49 % (56/114) were from females. The distribution in terms of month of sampling, age of patients and HRV types is shown in Fig. 1. The age ranged from 2 weeks to 90 years: 27 % were <12 months, 24 % were 1.0–9.9 years and 49 % were 10.0–90 years of age. In patients

Table 1 Human rhinovirus (HRV) types identified during four seasons

HRV type	Total	06/07	07/08	08/09	09/10
A7	2				2
A10	3			2	1
A12	3		2		1
A13	1				1
A20	2			2	
A28	1	1			
A30	1			1	
A33	3		1	2	
A34	3		1	2	
A36	2		2		
A39	1			1	
A41	1				1
A43	2			1	1
A46	1			1	
A47	2			2	
A49	3			2	1
A53	3			3	
A55	2			2	
A56	5	2		3	
A58	2		1	1	
A59	2		1		1
A60	2		1	1	
A63	2			1	1
A65	1	1			
A66	1	1			
A67	2			2	
A68	2			2	
A71	1	1			
A75	1		1		
A77	2		1		1
A78	3	1	1	1	
A80	1	1			
A82	1				1
B6	2		2		
B26	1				1
B42	1		1		
B70	1			1	
B72	1		1		
B84	1			1	
B91	2	1			1
B92	1				1
B97	1				1
C?	1			1	
C7?	2		1		1
C8?	1				1
C9	7	2		4	1
C11	1				1
C12	2		1		1
C14	2			1	1
C15	2			2	
C16	1		1		
C18	1			1	
C22	1		1		
C24	1			1	
C25	2			2	
C26	1			1	
C29?	1			1	
C35	2			2	
C38	2		1	1	
C39	1			1	
C43	3				3
C44	2				2
C46?	1		1		

HRV-C sequences with >15 % divergence from reference sequences are indicated by a question mark

The 09/10 season includes four cases from September 2010

aged 10 years or more, HRV-B was relatively more common (17 %) and HRV-C was relatively less common (24 %) than in those younger than 10 years of age (3.5 % and 42 %, respectively). In samples taken in September, there was a predominance of HRV-A and HRV-B in samples from patients who were more than 10 years old.

Seasonal distribution of types

Each season, defined as September through May in the following year, several HRV types were observed. In 2006/2007, we found nine A types, one B type and two C types; in 2007/2008, we found 12 A types, three B types and seven C types; in 2008/2009, we found 29 A types, two B types and 17 C types; in 2009/2010, we found 12 A types, three B types and 12 C types. Some types were found across several seasons, such as A78 and C9, which appeared during three consecutive seasons, and A10, A12, A33, A34, A43, A49, A56, A58, A59, A60, A63, A77, A78, B91, C7, C12, C14, C35 and C38, which appeared during two seasons.

Phylogenetic analysis of nucleotide sequences

The mean nucleotide difference was 39.3 % between HRV-A and HRV-B, 38.5 % between HRV-A and HRV-C, and 40.2 % between HRV-B and HRV-C. The variability within HRV-C strains was greater (24.4 %) than within HRV-A (20.3 %, $p<0.0001$) and HRV-B (21.1 %, $p=0.0002$) strains.

The phylogenetic tree in Fig. 2 shows all the HRV sequences identified in our investigation, along with

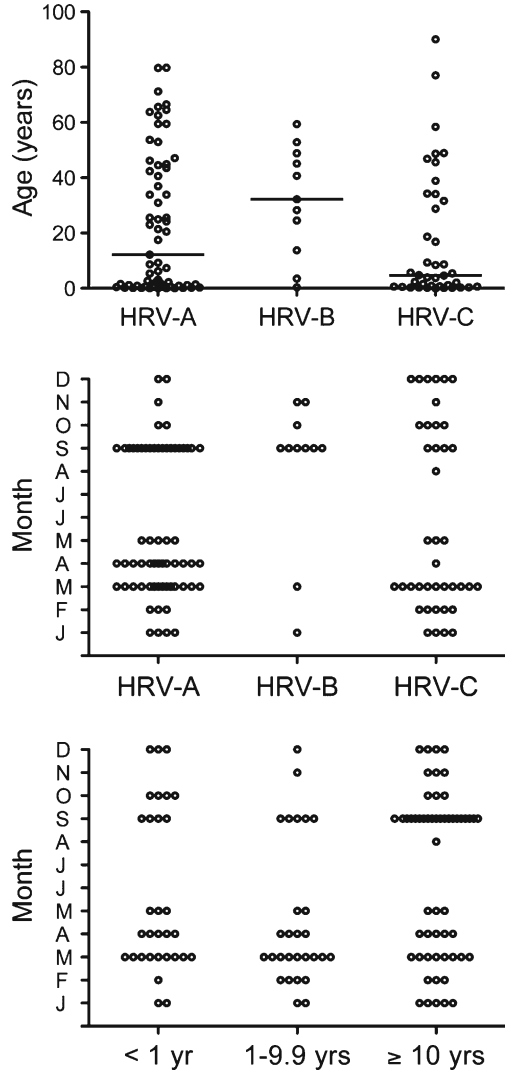


Fig. 1 Distribution of patient age, virus type and sample collection time point for 112 human rhinovirus (HRV) infections

reference sequences representing the various HRV types that were observed. The tree reveals that some of the HRV types appeared during two or three seasons, suggesting that these types might circulate in the population over long time periods. To further explore this, we constructed phylogenetic trees for each of these types, including sequences from our patients in comparison with ≈10 related sequences from GenBank retrieved by BLAST search. As shown in Fig. 3, some of these trees, indeed, show examples of strains or lineages of HRV that circulated in our population for 5–12 months, as indicated by a greater genetic similarity between them than with the most closely related sequences in GenBank. This analysis indicated that an HRV-A10 strain remained for 4 months, A33 for 12 months, A34 for 5 months, A49 for 4 months, A56 for 2 months, A60 for 7 months, C9 for 9 months, C14 for 10 months and C43 for 3 months. In other cases (Fig. 3b, e, j), strains belonging to the same type were more similar to database sequences than to each other. These trees also demonstrate that, for most of the HRV strains from our patients, there were several closely related sequences found in GenBank. The majority of the related sequences had been collected during the same year as our sequence, or the previous or following years.

Putative new types

One HRV-B (DE8-2793) and six HRV-C sequences (DE9-2878, DE10-2553, DE7-5952, DE9-9782, DE9-2601 and DE8-2155) showed less than 85 % nucleotide similarity with the most similar reference sequences (B6, C7, C8, C29 and C46), suggesting that they might represent new types. For each of these cases, there was at least one published sequence with 91–98 % similarity (in VP4/VP2), but the HRV types of these published sequences has not been defined because the strains have not been analysed in VP1, which is required for type assignment [23].

Discussion

In contrast to infections with most other enveloped respiratory viruses, such as influenza virus or RSV, HRV infections are common across all seasons, with a slightly lower incidence during summer and a peak in early autumn. To some extent, the seasonal pattern of HRV infections prevailing throughout the year might be explained by the great genetic diversity between and within HRV types. Thus, each HRV type might cause a restricted outbreak similar to most other viral ARTI agents. If this was true, HRV types would circulate in the local population during a limited time span, and over a four-year period, one would, by sequencing studies, be able to observe a large number of successive epidemics caused by different HRV types. It might be anticipated that a very large number of HRV strains would have to be sequenced in order to clarify this issue properly. Still, despite the limited sample size, this possibility was, to some extent, supported by the present study. Firstly, many HRV types were observed during each season and different types appeared over time. Secondly, the application of a BLAST search strategy on each HRV type that re-appeared during several seasons made it possible to show that such cases were sometimes caused by different strains of the same HRV type, with each strain closely related to strains observed in other parts of the world at approximately the same time. For example, two cases of HRV-A12 presenting in March 2008 were unrelated to another HRV-A12 strain found in April 2010, and one HRV-A10 strain appearing in May 2009 was more similar to strains from Italy, USA, Japan and Switzerland than to another HRV-A10 strain found in one of our samples from the same month. These

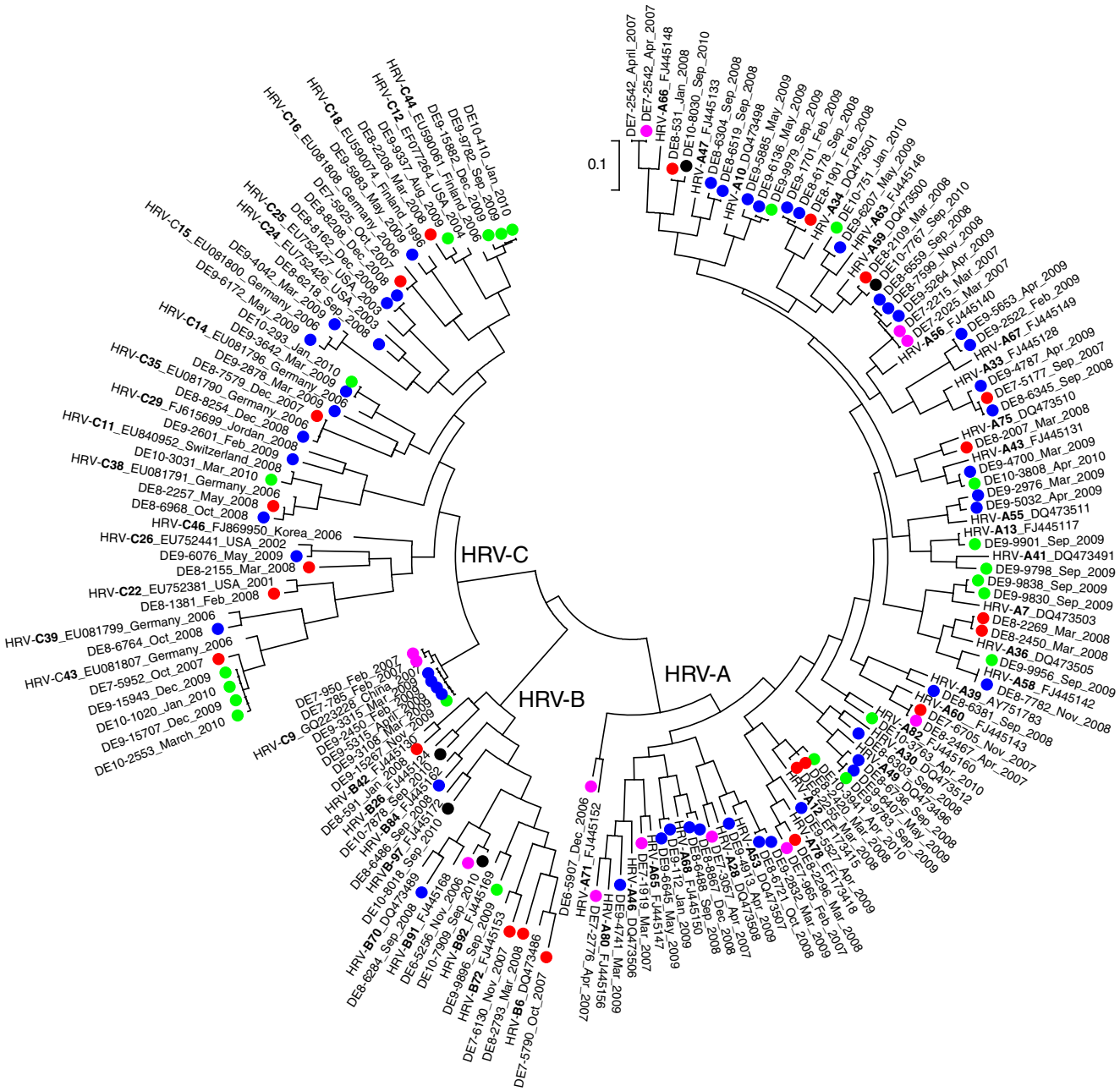
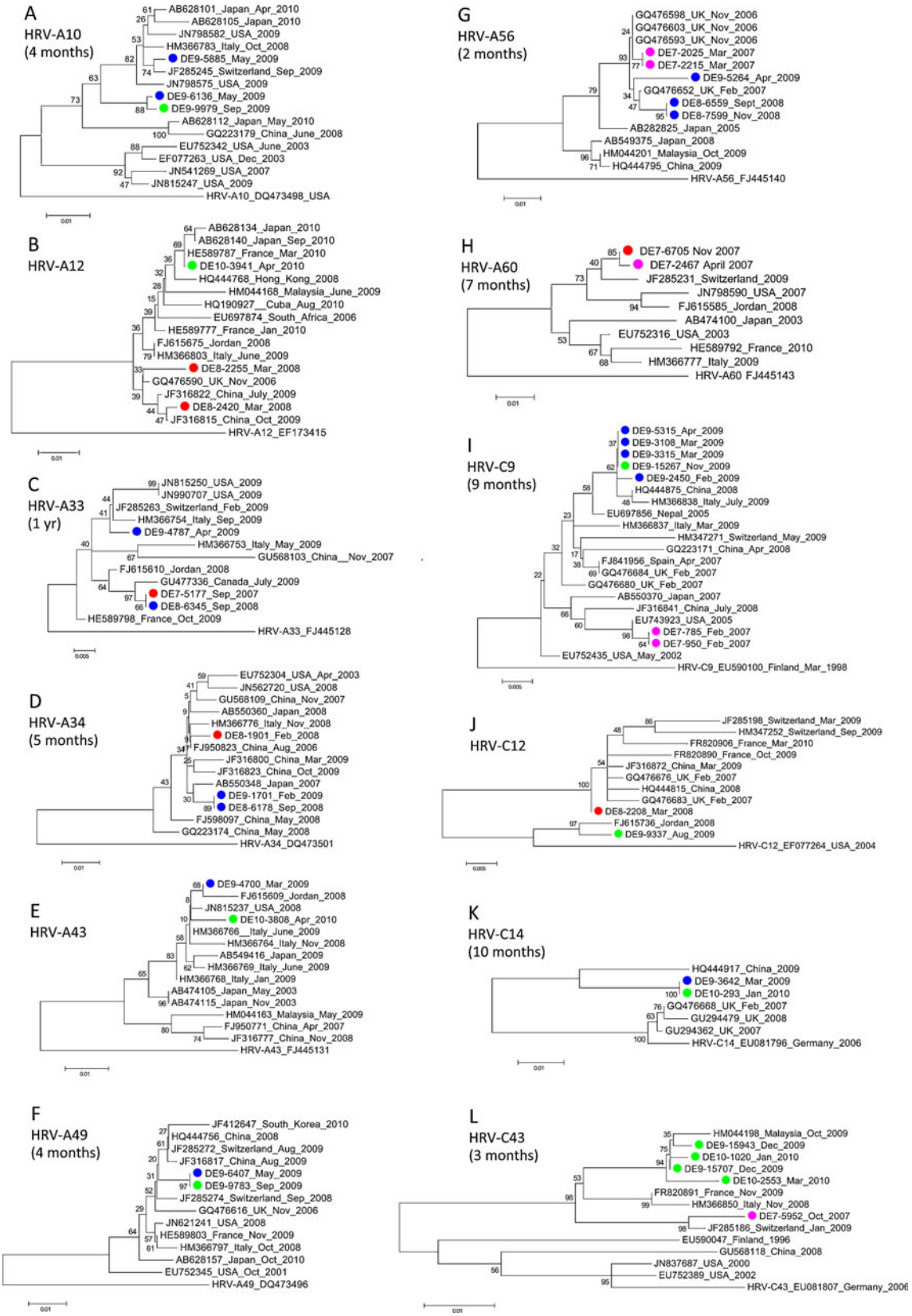


Fig. 2 Phylogenetic tree by maximum-likelihood analysis of 112 HRV sequences from the present study and database reference sequences (*in bold*). The coloured dots indicate the sampling season: *pink*, 2006/2007; *red*, 2007/2008; *blue*, 2008/2009; *green*, 2009/2010; *black* 2010

findings support that HRV strains may appear locally in a time-limited fashion, similarly to outbreak patterns typical for influenza A and RSV [11].

However, other HRV strains appeared to circulate in our population over relatively long time periods, in some cases from spring to autumn. For example, one HRV-C9 appearing in November 2009 was identical to other C9 strains from March 2009, and an HRV-A33 sequence from September 2008 was identical to another one found one year earlier. These cases demonstrate that HRV epidemics may be more long lasting than influenza A and RSV outbreaks, which typically wane after 4–8 weeks, when the number of susceptible individuals have declined below a critical level. The explanation to the more extended duration of HRV epidemics is probably multi-factorial. Firstly, the clinical presentation of HRV infections is, overall, milder compared with influenza A, and HRV have been identified in significant proportions of individuals without ongoing respiratory symptoms [15, 24, 25]. Thus, HRV-infected subjects are more likely to expose other people to the infection. Secondly, HRV are shed during longer time periods, typically 2–3 weeks, as compared with less than 1 week for


A
HRV-A10
(4 months)
AB628101_Japan_Apr_2010
AB628105_Japan_2010
JN798582_USA_2009
HM366783_Italy_Oct_2008
DE9-5885_May_2009
JF285245_Switzerland_Sep_2009
JN798575_USA_2009
DE9-6136_May_2009
DE9-9979_Sep_2009
AB628112_Japan_May_2010
GQ223179_China_June_2008
EU752342_USA_June_2003
EF077263_USA_Dec_2003
JN541269_USA_2007
JN815247_USA_2009
HRV-A10_DQ473498_USA
B
HRV-A12
AB628134_Japan_2010
AB628140_Japan_Sep_2010
HE589787_France_Mar_2010
DE10-3941_Apr_2010
HQ444768_Hong_Kong_2008
HM044168_Malaysia_June_2009
HQ190927__Cuba_Aug_2010
EU697874_South_Africa_2006
HE589777_France_Jan_2010
FJ615675_Jordan_2008
HM366803_Italy_June_2009
DE8-2255_Mar_2008
GQ476590_UK_Nov_2006
JF316822_China_July_2009
DE8-2420_Mar_2008
JF316815_China_Oct_2009
HRV-A12_EF173415
C
HRV-A33
(1 yr)
JN815250_USA_2009
JN990707_USA_2009
JF285263_Switzerland_Feb_2009
HM366754_Italy_Sep_2009
DE9-4787_Apr_2009
HM366753_Italy_May_2009
GU568103_China__Nov_2007
FJ615610_Jordan_2008
GU477336_Canada_July_2009
DE7-5177_Sep_2007
DE8-6345_Sep_2008
HE589798_France_Oct_2009
HRV-A33_FJ445128
D
HRV-A34
(5 months)
EU752304_USA_Apr_2003
JN562720_USA_2008
GU568109_China_Nov_2007
AB550360_Japan_2008
HM366776_Italy_Nov_2008
DE8-1901_Feb_2008
FJ950823_China_Aug_2006
JF316800_China_Mar_2009
JF316823_China_Oct_2009
AB550348_Japan_2007
DE9-1701_Feb_2009
DE8-6178_Sep_2008
FJ598097_China_May_2008
GQ223174_China_May_2008
HRV-A34_DQ473501
E
HRV-A43
DE9-4700_Mar_2009
FJ615609_Jordan_2008
JN815237_USA_2008
DE10-3808_Apr_2010
HM366766__Italy_June_2009
HM366764_Italy_Nov_2008
AB549416_Japan_2009
HM366769_Italy_June_2009
HM366768_Italy_Jan_2009
AB474105_Japan_May_2003
AB474115_Japan_Nov_2003
HM044163_Malaysia_May_2009
FJ950771_China_Apr_2007
JF316777_China_Nov_2008
HRV-A43_FJ445131
F
HRV-A49
(4 months)
JF412647_South_Korea_2010
HQ444756_China_2008
JF285272_Switzerland_Aug_2009
JF316817_China_Aug_2009
DE9-6407_May_2009
DE9-9783_Sep_2009
JF285274_Switzerland_Sep_2008
GQ476616_UK_Nov_2006
JN621241_USA_2008
HE589803_France_Nov_2009
HM366797_Italy_Oct_2008
AB628157_Japan_Oct_2010
EU752345_USA_Oct_2001
HRV-A49_DQ473496
G
HRV-A56
(2 months)
GQ476598_UK_Nov_2006
GQ476603_UK_Nov_2006
GQ476593_UK_Nov_2006
DE7-2025_Mar_2007
DE7-2215_Mar_2007
DE9-5264_Apr_2009
GQ476652_UK_Feb_2007
DE8-6559_Sept_2008
DE8-7599_Nov_2008
AB282825_Japan_2005
AB549375_Japan_2008
HM044201_Malaysia_Oct_2009
HQ444795_China_2009
HRV-A56_FJ445140
H
HRV-A60
(7 months)
DE7-6705 Nov 2007
DE7-2467 April 2007
JF285231_Switzerland_2009
JN798590_USA_2007
FJ615585_Jordan_2008
AB474100_Japan_2003
EU752316_USA_2003
HE589792_France_2010
HM366777_Italy_2009
HRV-A60 FJ445143
I
HRV-C9
(9 months)
DE9-5315_Apr_2009
DE9-3108_Mar_2009
DE9-3315_Mar_2009
DE9-15267_Nov_2009
DE9-2450_Feb_2009
HQ444875_China_2008
HM366838_Italy_July_2009
EU697856_Nepal_2005
HM366837_Italy_Mar_2009
HM347271_Switzerland_May_2009
GQ223171_China_Apr_2008
FJ841956_Spain_Apr_2007
GQ476684_UK_Feb_2007
GQ476680_UK_Feb_2007
AB550370_Japan_2007
JF316841_China_July_2008
EU743923_USA_2005
DE7-785_Feb_2007
DE7-950_Feb_2007
EU752435_USA_May_2002
HRV-C9_EU590100_Finland_Mar_1998
J
HRV-C12
JF285198_Switzerland_Mar_2009
HM347252_Switzerland_Sep_2009
FR820906_France_Mar_2010
FR820890_France_Oct_2009
JF316872_China_Mar_2009
GQ476676_UK_Feb_2007
HQ444815_China_2008
GQ476683_UK_Feb_2007
DE8-2208_Mar_2008
FJ615736_Jordan_2008
DE9-9337_Aug_2009
HRV-C12_EF077264_USA_2004
K
HRV-C14
(10 months)
HQ444917_China_2009
DE9-3642_Mar_2009
DE10-293_Jan_2010
GQ476668_UK_Feb_2007
GU294479_UK_2008
GU294362_UK_2007
HRV-C14_EU081796_Germany_2006
L
HRV-C43
(3 months)
HM044198_Malaysia_Oct_2009
DE9-15943_Dec_2009
DE10-1020_Jan_2010
DE9-15707_Dec_2009
DE10-2553_Mar_2010
FR820891_France_Nov_2009
HM366850_Italy_Nov_2008
DE7-5952_Oct_2007
JF285186_Switzerland_Jan_2009
EU590047_Finland_1996
GU568118_China_2008
JN837687_USA_2000
EU752389_USA_2002
HRV-C43_EU081807_Germany_2006

◀ **Fig. 3** Phylogenetic tree by maximum-likelihood analysis of HRV types appearing for two or more seasons. For trees that support persistence of strains, the duration of this persistence is given in *parentheses*. The bootstrap values indicate how frequently the tree topology appeared when 1000 replicate trees were analysed. The coloured dots indicate the season: *pink*, 2006/2007; *red*, 2007/2008; *blue*, 2008/2009; *green*, 2009/2010; *black* 2010

influenza A [26], probably resulting in a higher likelihood of spreading of the infection. Immunocompetent individuals may even shed the same strain of HRV for more than 4 weeks [27–29], and in immunocompromised individuals, shedding for as long as 27 months has been observed [30]. Thirdly, the robust unenveloped virion structure of HRV may increase the likelihood of indirect transmission via surfaces [30].

Our study differs from most previous reports by having a longer observation period and by not being performed in a defined patient group, such as hospitalized children, but representing a judgement sample of rhinoviruses in different types of patients seeking medical care during a defined time period in a fairly well-defined area. Still, the observed proportions of HRV types (57 % HRV-A, 10 % HRV-B and 33 % HRV-C) largely agrees with recent reports from UK, Italy, France and Japan (47–62 % HRV-A, 6–9 % HRV-B and 30–47 % HRV-C [17, 19, 20, 31]. The total number of observed types in the present study (33 HRV-A, nine HRV-B and 21 HRV-C types) was higher than the 22 HRV-A, six HRV-B and 15 HRV-C types that were reported by Wisdom et al. over a period of 6 months but less than the 71 HRV-A, ten HRV-B and 47 HRV-C reported over 26 months by Tapparel et al. [32].

In the present study, phylogeny based on VP4/VP2 sequences was used for classification. This has previously been shown to agree well with the more reliable typing by phylogeny of VP1 sequences, and for HRV-A and HRV-B, a good correlation has been documented for VP1 sequence phylogeny and serological classification [18, 33, 34]. For HRV-C, in the absence of serological typing techniques, the classification of HRV is based solely on sequence comparison, and a nucleotide divergence of more than 13 % in VP1 has been proposed to be used to define new HRV-C types [23]. The VP4/VP2 region can also be used to classify HRV-C, but it is not considered to be sufficient for the identification of new types [23]. Thus, additional sequencing of VP1 is required in order to clarify if any of the five HRV-C sequences (and one HRV-B sequence) that were more than 15 % different in the VP4/VP2 region might represent a new HRV type.

Our multiple PCR contains separate reaction mixtures for HRV and enterovirus, in order to allow distinction between these agents. Despite this, enteroviruses were found in two cases that were positive by the HRV real-time PCR. This reactivity is explained by the genetic similarity between HRV and enterovirus, but these cases were reactive with lower Ct values by the enterovirus component of the real-time PCR panel. The observation that enteroviruses may cause ARTI and be detected in nasal swabs is well known, and the relative frequency of enterovirus infections found in our study is similar to what others have reported [18].

In summary, we observed a wide spectrum of HRV types, changing over time, which may contribute to the seasonal pattern of HRV infections throughout the year. HRV in our patients were often closely related to published sequences from distant locations, suggesting that HRV epidemics may be highly globalised. However, some of the results demonstrated the presence of the same HRV types across extended time periods, which calls for further studies on the impact of the duration of viral shedding, virion structure robustness and insufficient immune responses in order to explain why HRV is the most common ARTI agent worldwide.

Acknowledgements This research was funded by the Region Västra Götaland Research Funds (VGFOUREG-82811, ALFGBG-217671).

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Bellei N, Carraro E, Perosa A, Watanabe A, Arruda E, Granato C (2008) Acute respiratory infection and influenza-like illness viral etiologies in Brazilian adults. J Med Virol 80:1824–1827
2. Arola M, Ziegler T, Ruuskanen O, Mertsola J, Näntö-Salonen K, Halonen P (1988) Rhinovirus in acute otitis media. J Pediatr 113:693–695
3. Johnstone J, Majumdar SR, Fox JD, Marrie TJ (2008) Viral infection in adults hospitalized with community-acquired pneumonia: prevalence, pathogens, and presentation. Chest 134:1141–1148
4. Tan WC (2005) Viruses in asthma exacerbations. Curr Opin Pulm Med 11:21–26
5. Bertino JS (2002) Cost burden of viral respiratory infections: issues for formulary decision makers. Am J Med 112(Suppl 6A):42S–49S
6. Arden KE, McErlean P, Nissen MD, Sloots TP, Mackay IM (2006) Frequent detection of human rhinoviruses, paramyxoviruses, coronaviruses, and bocavirus during acute respiratory tract infections. J Med Virol 78:1232–1240
7. McErlean P, Shackelton LA, Andrews E, Webster DR, Lambert SB, Nissen MD, Sloots TP, Mackay IM (2008) Distinguishing molecular features and clinical characteristics of a putative new rhinovirus species, human rhinovirus C (HRV C). PLoS One 3: e1847
8. Renwick N, Schweiger B, Kapoor V, Liu Z, Villari J, Bullmann R, Miething R, Briese T, Lipkin WI (2007) A recently identified rhinovirus genotype is associated with severe respiratory-tract infection in children in Germany. J Infect Dis 196:1754–1760
9. Wisdom A, Kutkowska AE, McWilliam Leitch EC, Gaunt E, Templeton K, Harvala H, Simmonds P (2009) Genetics, recombination and clinical features of human rhinovirus species C (HRV-C) infections; interactions of HRV-C with other respiratory viruses. PLoS One 4:e8518
10. Lau SK, Yip CC, Tsoi HW, Lee RA, So LY, Lau YL, Chan KH, Woo PC, Yuen KY (2007) Clinical features and complete genome characterization of a distinct human rhinovirus (HRV) genetic

cluster, probably representing a previously undetected HRV species, HRV-C, associated with acute respiratory illness in children. J Clin Microbiol 45:3655–3664
11. Olofsson S, Brittain-Long R, Andersson LM, Westin J, Lindh M (2011) PCR for detection of respiratory viruses: seasonal variations of virus infections. Expert Rev Anti Infect Ther 9:615–626
12. Brittain-Long R, Andersson LM, Olofsson S, Lindh M, Westin J (2012) Seasonal variations of 15 respiratory agents illustrated by the application of a multiplex polymerase chain reaction assay. Scand J Infect Dis 44:9–17
13. Monto AS (2002) The seasonality of rhinovirus infections and its implications for clinical recognition. Clin Ther 24:1987–1997
14. Weigl JA, Puppe W, Meyer CU, Berner R, Forster J, Schmitt HJ, Zepp F (2007) Ten years' experience with year-round active surveillance of up to 19 respiratory pathogens in children. Eur J Pediatr 166:957–966
15. Winther B, Hayden FG, Hendley JO (2006) Picornavirus infections in children diagnosed by RT-PCR during longitudinal surveillance with weekly sampling: association with symptomatic illness and effect of season. J Med Virol 78:644–650
16. Fox JP, Cooney MK, Hall CE (1975) The Seattle virus watch. V. Epidemiologic observations of rhinovirus infections, 1965–1969, in families with young children. Am J Epidemiol 101:122–143
17. Piralla A, Rovida F, Campanini G, Rognoni V, Marchi A, Locatelli F, Gerna G (2009) Clinical severity and molecular typing of human rhinovirus C strains during a fall outbreak affecting hospitalized patients. J Clin Virol 45:311–317
18. Savolainen C, Mulders MN, Hovi T (2002) Phylogenetic analysis of rhinovirus isolates collected during successive epidemic seasons. Virus Res 85:41–46
19. Wisdom A, Leitch EC, Gaunt E, Harvala H, Simmonds P (2009) Screening respiratory samples for detection of human rhinoviruses (HRVs) and enteroviruses: comprehensive VP4–VP2 typing reveals high incidence and genetic diversity of HRV species C. J Clin Microbiol 47:3958–3967
20. Henquell C, Mirand A, Deusebis AL, Regagnon C, Archimbaud C, Chambon M, Bailly JL, Gourdon F, Hermet E, Dauphin JB, Labbé A, Peigue-Lafeuille H (2012) Prospective genotyping of human rhinoviruses in children and adults during the winter of 2009–2010. J Clin Virol 53:280–284
21. Brittain-Long R, Nord S, Olofsson S, Westin J, Anderson LM, Lindh M (2008) Multiplex real-time PCR for detection of respiratory tract infections. J Clin Virol 41:53–56
22. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731–2739
23. Simmonds P, McIntyre C, Savolainen-Kopra C, Tapparel C, Mackay IM, Hovi T (2010) Proposals for the classification of human rhinovirus species C into genotypically assigned types. J Gen Virol 91:2409–2419
24. Peltola V, Waris M, Osterback R, Susi P, Ruuskanen O, Hyypiä T (2008) Rhinovirus transmission within families with children: incidence of symptomatic and asymptomatic infections. J Infect Dis 197:382–389
25. van der Zalm MM, Wilbrink B, van Ewijk BE, Overduin P, Wolfs TF, van der Ent CK (2011) Highly frequent infections with human rhinovirus in healthy young children: a longitudinal cohort study. J Clin Virol 52:317–320
26. Brittain-Long R, Westin J, Olofsson S, Lindh M, Andersson LM (2011) Access to a polymerase chain reaction assay method targeting 13 respiratory viruses can reduce antibiotics: a randomised, controlled trial. BMC Med 9:44
27. Jartti T, Lehtinen P, Vuorinen T, Koskenvuo M, Ruuskanen O (2004) Persistence of rhinovirus and enterovirus RNA after acute respiratory illness in children. J Med Virol 72:695–699
28. Pitkäranta A, Roivainen M, Blomgren K, Peltola J, Kaijalainen T, Räty R, Ziegler T, Rönkkö E, Hatakka K, Korpela R, Poussa T, Leinonen M, Hovi T (2006) Presence of viral and bacterial pathogens in the nasopharynx of otitis-prone children. A prospective study. Int J Pediatr Otorhinolaryngol 70:647–654
29. Rosenbaum MJ, De Berry P, Sullivan EJ, Pierce WE, Mueller RE, Peckinpaugh RO (1971) Epidemiology of the common cold in military recruits with emphasis on infections by rhinovirus types 1A, 2, and two unclassified rhinoviruses. Am J Epidemiol 93:183–193
30. Tapparel C, Cordey S, Van Belle S, Turin L, Lee WM, Regamey N, Meylan P, Mühlemann K, Gobbini F, Kaiser L (2009) New molecular detection tools adapted to emerging rhinoviruses and enteroviruses. J Clin Microbiol 47:1742–1749
31. Fujitsuka A, Tsukagoshi H, Arakawa M, Goto-Sugai K, Ryo A, Okayama Y, Mizuta K, Nishina A, Yoshizumi M, Kaburagi Y, Noda M, Tashiro M, Okabe N, Mori M, Yokota S, Kimura H (2011) A molecular epidemiological study of respiratory viruses detected in Japanese children with acute wheezing illness. BMC Infect Dis 11:168
32. Tapparel C, Cordey S, Junier T, Farinelli L, Van Belle S, Soccal PM, Aubert JD, Zdobnov E, Kaiser L (2011) Rhinovirus genome variation during chronic upper and lower respiratory tract infections. PLoS One 6:e21163
33. Laine P, Blomqvist S, Savolainen C, Andries K, Hovi T (2006) Alignment of capsid protein VP1 sequences of all human rhinovirus prototype strains: conserved motifs and functional domains. J Gen Virol 87:129–138
34. Ledford RM, Patel NR, Demenczuk TM, Watanyar A, Herbertz T, Collett MS, Pevear DC (2004) VP1 sequencing of all human rhinovirus serotypes: insights into genus phylogeny and susceptibility to antiviral capsid-binding compounds. J Virol 78:3663–3674