medRxiv preprint doi: https://doi.org/10.1101/2020.03.09.20033019.this version posted March 19, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

Molecular epidemiology of human rhinovirus from one-year surveillance within a school setting in rural coastal Kenya

Martha M. Luka[1,2,*]; Everlyn Kamau[1]; Irene Adema[1]; Patrick K. Munywoki[1]; Grieven P. Otieno[1]; Elijah Gicheru[1]; Alex Gichuki[1]; Nelson Kibinge[1]; Charles N. Agoti[1,2]; D. James Nokes[1,3]

Author Affiliations:

1. Epidemiology and Demography Department, KEMRI-Wellcome Trust Research Programme, Centre for Geographic Medicine Research – Coast, Kilifi, Kenya.
2. Department of Public Health, Pwani University, Kilifi, Kenya.
3. School of Life Sciences and Zeeman Institute for Systems Biology and Infectious Disease Epidemiology Research (SBIDER), University of Warwick, Coventry, United Kingdom.

[*] **Corresponding author:** mluka@kemri-wellcome.org

Summary points: We describe the molecular epidemiology of human rhinovirus (HRV) within a school setting over one-year in rural coastal Kenya. A high diversity of HRV infections was observed across all classes with evidence of introduction and transmission of 47 different genotypes.

Keywords: Human rhinovirus, transmission, molecular epidemiology, school-going children, Kenya

medRxiv preprint doi: https://doi.org/10.1101/2020.03.09.20033019.this version posted March 19, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

ABSTRACT

Background: Human rhinovirus (HRV) is the most common cause of the common cold but may also lead to more severe respiratory illness in vulnerable populations. The epidemiology and genetic diversity of HRV within a school setting have not been described.

Objective: To characterise HRV molecular epidemiology among children attending primary school in a rural location of Kenya.

Methods: Between May 2017 to April 2018, over three school terms, we collected 1859 nasopharyngeal swabs (NPS) from pupils and teachers with symptoms of acute respiratory infection in a public primary school in Kilifi County, coastal Kenya. The samples were tested for HRV using real-time RT-PCR. HRV positive samples were sequenced in the VP4/VP2 coding region for species and genotype classification.

Results: A total of 307 NPS (16.4%) from 164 individuals were HRV positive, and 253 (82.4%) were successfully sequenced. The proportion of HRV in the lower primary classes was higher (19.8%) than upper primary classes (12.2%), *p*-value <0.001. HRV-A was the most common species (134/253, 53.0%), followed by HRV-C (73/253, 28.9%) and HRV-B (46/253, 18.2%). Phylogenetic analysis identified 47 HRV genotypes. The most common genotypes were A2 and B70. Numerous (up to 22 in one school term) genotypes circulated simultaneously, there was no individual re-infection with the same genotype, and no genotype was detected in all three school terms.

Conclusion: HRV was frequently detected among school-going children with mild ARI symptoms, and particularly in the younger age groups (<5-year-olds). Multiple HRV introductions were observed characterised by the considerable genotype diversity.

medRxiv preprint doi: https://doi.org/10.1101/2020.03.09.20033019.this version posted March 19, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

INTRODUCTION

Human rhinovirus (HRV) is a frequently detected viral respiratory pathogen [1] associated with the common cold [2,3], lower respiratory tract infections [4] and asthma development and exacerbation [5,6]. Although the majority of HRV cases are mild and self-limiting, they contribute to substantial economic losses through missed school and workdays [7,8]. HRV is a common reason for prescribing antibiotics [9], potentially contributing to antibiotic resistance. The virus is transmitted via inhalation of contaminated aerosols and close contact with infected persons or surfaces [10], and prolonged periods of close contact increase the transmission efficiency [11]. Children, the elderly and those with pre-existing respiratory conditions have the highest HRV burden in the community [12–14].

HRV is a positive sense, single-stranded RNA virus, classified under the genus *Enterovirus* (family *Picornaviridae)* with a genome approximately 7.2 kb long. It is characterised by high genetic diversity and an associated high antigenic diversity [15], frustrating vaccine development efforts. There are 169 HRV genotypes distributed across three species: 80 HRV-A genotypes, 32 HRV-B genotypes and 57 HRV-C genotypes [16]. HRV is non-enveloped and has a spherical capsid with a diameter of about 30 nm which constitutes of 60 copies of each of the four viral proteins: VP4, VP2, VP3 and VP1 [17], in that order. Serotype assignment was initially done using monoclonal antibodies by virus neutralisation assays for species A and B [18,19]. However, HRV-C, discovered in 2006 [20], cannot propagate in conventional cell lines limiting serotyping as a classification method [21,22]. Molecular classification is based on the nucleotide sequence of either the 5' noncoding region, VP1 or the VP4/2 genome region [22–24].

Children constitute a significant reservoir of HRV [25]. In sub-Saharan Africa, little has been done to investigate the patterns and mechanisms of transmission of HRV in schools and to understand the extent to which school settings contribute to transmission in the community. This is despite

medRxiv preprint doi: https://doi.org/10.1101/2020.03.09.20033019.this version posted March 19, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

that primary school children have the highest contact rates compared to other age-groups in the community [26] and play a significant role in infection transmission to their younger siblings [27,28]. Design of effective intervention strategies against HRV relies on our knowledge of transmission dynamics of the virus in different social networks and population structures [8,29]. No study has yet explored the diversity and dynamics of HRV infections among school children. This has limited our understanding of the transmission patterns of HRV in this social grouping and the community as a whole.

This study investigated HRV infections in a school setting in rural coastal Kenya by sequence analysis of the VP4/2 junction to describe the frequency, diversity and temporal occurrence of HRV.

METHODS

Study area and design. An epidemiological surveillance study was conducted in a rural school located within the Kilifi Health and Demographic Surveillance System (KHDSS) in Kenya [30] to characterise the occurrence of respiratory viruses. The study design is described in detail elsewhere [31]. Briefly, the school offers both early childhood development education and primary school education. Pupils and teachers from all classes were enrolled in the study, which took place between May 2017 and April 2018. Pupils were divided into two main groups: the lower primary comprising of day care, kindergarten (KG) levels 1 to 3 and grade one (*n*=5 classes, age range 3-12 years); and the upper primary comprising grades two to eight (*n*=7 classes, age range 7-20 years).

Nasopharyngeal swabs (NPS) were collected from individuals who had at least one of the following acute respiratory illness (ARI) symptoms: cough, sore throat or runny nose. A maximum

medRxiv preprint doi: https://doi.org/10.1101/2020.03.09.20033019.this version posted March 19, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

of 8 samples per class was collected from the lower primary group per week, while a maximum of 4 samples per class was collected from the upper primary group per week. A maximum of 3 samples was collected from the teachers per week. We collected more samples from the lower primary due to the perceived critical role of this age-groups in childhood infectious diseases and hence the need to reduce the level of uncertainty in the estimated risk in this age-groups. Samples were collected in viral transport media (VTM) and transported in cool boxes to the KEMRI-Wellcome Trust Research Programme laboratory where they were stored at -80°C prior to screening. Sampling was suspended during school holidays: months of August, November and December 2017 and from 6th April 2018, which marked the end of the study.

An informed written parental consent for children under the age of eighteen years or individual consent for adults was obtained prior to sample collection. In addition, children whose parents consented were asked for individual assent to participate. Ethical approval was provided by the KEMRI-Scientific Ethics Review Unit (KEMRI-SERU #3332) and the University of Warwick Biomedical and Scientific Research Ethics Committee (BSREC #REGO_2016-1858).

RNA extraction and rRT-PCR. RNA was extracted from 140 µl of the collected sample using QIAamp® 96 Virus QIAcube HT kit (Qiagen, United Kingdom), according to the manufacturer's instructions. All samples were tested for HRV using an in-house multiplexed real-time reverse-transcription PCR (rRT-PCR) with a QuantiFast® Multiplex RT-PCR kit (Qiagen, United Kingdom) [32–34]. A sample was considered HRV positive if the rRT-PCR cycle threshold (*Ct*) value was <35.

VP4/2 amplification and sequencing. VP4/2 sequencing was used to assign species and genotypes. A genomic region approximately 549 nucleotides and consisting of a hypervariable region of the 5'UTR, the complete VP4 and partial VP2 gene region was amplified for all HRV

medRxiv preprint doi: https://doi.org/10.1101/2020.03.09.20033019.this version posted March 19, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

positive samples using a One-Step RT-PCR kit (QIAGEN®) as previously described [35,36]. PCR products were purified using the MinElute® PCR purification kit (Qiagen, United Kingdom) and sequenced with the respective forward and reverse primers in a BigDye® terminator version 3.1 (Applied Biosystems, USA) reaction and analysed in an ABI 3130xl genetic analyser.

Sequence analysis. Raw sequence reads were quality-checked, trimmed, edited and assembled to contigs of length 420 nucleotides using Sequencher version 5.4.6 (www.genecodes.com). Alignments were prepared using MAFFT version 7.271 [37]. IQ-TREE version 1.6.0 [38] was used to estimate the best fit model and infer Maximum Likelihood (ML) trees. Phylogenetic trees were generated with bootstrapping of 1000 iterations. Pairwise nucleotide p-distances were calculated using MEGA version 7.0.21 [39]. Genotype assignment was based on the phylogenetic clustering (bootstrap value >80%) on ML trees and pairwise genetic distances to prototype strains (https://www.picornaviridae.com/enterovirus/prototypes/prototypes.htm) as proposed (10.5% for HRV-A, 9.5% for HRV-B, and 10.5% for HRV-C) [22,23].

Intra-type diversity for genotypes with at least ten sequences/samples was studied by visualisation of the number of nucleotide substitutions to the earliest sampled sequence for each type. The substitution rate of the VP4/2 coding region in HRV had previously been estimated as 7×10^{-4} to 4×10^{-3} substitutions/site/year [40]. Using the upper evolutionary rate value translated to 1.68 nucleotide substitutions per year across the sequenced 420 nt segment. We therefore defined an intra-type variant as a sequence with >2 nucleotide differences from the index sequence. An intra-type variant had to be observed in at least two sequences to increase confidence that this would not be the result of sequencing error.

Data Analysis. Data analysis was conducted using STATA version 13 (STATA Corp Texas) and R version 3.6.1 (CRAN R Project). Categorical variables were summarised using frequencies and

medRxiv preprint doi: https://doi.org/10.1101/2020.03.09.20033019.this version posted March 19, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

percentages. HRV proportion for each class and respective 95% confidence intervals were defined as the number of HRV positive samples out of the total number of samples tested per class. The chi-square test for trend was used to check for linear trend in HRV proportion with an increasing hierarchy of classes in the school (from day care to teaching staff).

Definition of terms. We defined 'persistence' as the continued occurrence of the same genotype within the same school term. Detection of a genotype in a subsequent school term was considered a genotype recurrence. We defined 'frequent' genotypes as those that occurred in at least five samples, from more than two individuals, and further investigated their temporal occurrence and persistence. We defined 'individual HRV re-infection' as the acquisition of a new genotype or the detection of a previously acquired genotype in a subsequent school term. Individual detection with the same genotype in consecutive samples was considered a continuing infection.

Sequence data availability. Sequences generated by this study are available in GenBank under accession numbers MT177659-MT177911.

RESULTS

Baseline characteristics and HRV detection

The total number of samples collected between May 2017 and April 2018 was 1859, of which 307 (16.5%) tested positive for HRV. These HRV positives were collectively from 164 individuals: 160 pupils and four teachers. The mean age of the HRV positive pupils was 9.4 years, with a standard deviation of ±3.9. The most common ARI symptom recorded among the HRV positive cases (single sampling events) was nasal discharge (n=278, 90.6%), cough (n=226, 73.6%) and sore throat (n=62, 20.2%). Only a small proportion of those detected with HRV could identify a household member with ARI-like symptoms (n=43, 14.0%). Among the household members

medRxiv preprint doi: https://doi.org/10.1101/2020.03.09.20033019.this version posted March 19, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

identified, 26/43 (60%) were school-going siblings, with 18 (69%) of them attending the same school and the other eight (31%), a different school, **Table 1**. HRV circulation was detected throughout the three school terms under observation. Seasonal variations of HRV infections could not be identified due to breaks in sample collection during the school holidays. The lower primary had a higher HRV proportion compared to upper primary (19.8% versus 12.2%), *p*-value <0. 001, **Figure 1**.

HRV diversity

Amplification and sequencing of the VP4/2 genomic region were attempted on all HRV positive samples resulting in 82.4% (253/307) success. The unsuccessful samples either failed to amplify or had poor sequence quality. The resulting sequences were classified into 47 HRV genotypes: 24 HRV-A genotypes, seven HRV-Bs and 16 HRV-Cs. HRV-A was the most common species (134/253, 53.0%), followed by HRV-C (73/253, 28.9%) and HRV-B (46/253, 18.2%)**.** Some sequences violated the previously proposed genotype assignment thresholds, **Supplementary Table 1.** These were included in the analysis and classified with a suffix "-like" to the most similar known genotype (e.g. B48-like). The most frequent genotypes were A2 (24/253, 9.5%), B70 (22/253, 8.7%), A36 (16/253, 6.3%) and B48-like (16/253, 6.3%), **Figure 2**.

Temporal occurrence and clustering patterns

Numerous genotypes circulated simultaneously in the school with 22, 15 and 19 unique genotypes observed in term one, term two and term three, respectively. Nine genotypes recurred during the study period. Of the 22 that occurred in term one, two re-occurred in term two and four in term three; whereas of the 15 observed in term two, three recurred in term three. No genotype was

medRxiv preprint doi: https://doi.org/10.1101/2020.03.09.20033019.this version posted March 19, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

observed across all the three school terms. Four of the recurring genotypes (A13, A59, B48, C3) were detected in the same class, **Figure 3**A.

Twelve genotypes were observed as singletons (i.e. present in a single sample/individual). We observed that frequent genotypes (n=21) circulated averagely for 28 days (median 23 days). Five of the frequent genotypes recurred in a subsequent school term. The longest persisting genotype was B70 (n=22 samples) seen in 81 days. Genotype A2 (n=24 samples) was similarly frequent but persisted for only 16 days. Among the frequent genotypes, none was limited to the upper primary group, whereas A10, A28, A101 and C3 were observed only in the lower primary group. However, no frequent genotype was limited to only one school class, **Error! Reference source not found.**.

Individual infection patterns

Of the 164 HRV positive individuals, 62 (37.8%) contributed more than one positive sample. Three pupils, all from the lower primary group, presented with the most HRV positive samples (n=8) per person. The 253 successfully sequenced samples were collectively contributed by 144 individuals. Repeat HRV detections (n=109) were a combination of persistent infections (24/109) and re-infections (85/109). The number of genotypes per person ranged from one to five. About two-thirds of the individuals (98/144, 68.1%) had only one HRV genotype across the study period. Overall, the highest HRV diversity per person (5 genotypes) was observed in three pupils, all from the lower primary classes, **Table 2**. Time to re-infection varied greatly (13-307 days), with a median of 77 days. However, no individual was re-infected with the same genotype across the study period **Figure 3**B.

medRxiv preprint doi: https://doi.org/10.1101/2020.03.09.20033019.this version posted March 19, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

Intra-type genetic diversity

Eight genotypes had at least ten samples/sequences, and five of these occurred as a single variant throughout the study. The remaining three, A28, B48 and B70, had more than one variant. Genotype A28 had two variants simultaneously observed within the same school term, and both variants were detected only in the lower primary group. For B48, the second variant was observed as a genotype recurrence in a subsequent school term. Genotype B70 had the highest diversity, with three variants observed within one school term. The first B70 variant (13 samples) occurred across lower and upper primary as well as teaching staff, the second (4 samples) was first observed 49 days after the genotype's overall index sequence (all samples from KG class 1) and the third (5 samples) was first observed 69 days after the genotype index sequence, with 4/5 samples coming from the lower primary. No individual had more than one variant of the same genotype, **Figure 5**.

HRV among teaching staff

Thirty-two samples were collected from teachers, of which 5 (15.6%) were HRV positive from 4 individuals. Three HRV genotypes were identified: A2 (n=2), B70 (n=2) and B99 (n=1). The A2 and B70 genotypes were detected in teachers several days after their initial detection in the student population. The B99 sample from a teacher was the only case of this genotype identified during the entire study period suggesting that they acquired the infection from outside the school setting, and no onward transmission was observed.

DISCUSSION

This study describes the molecular epidemiology of HRV in a public primary school setting in rural coastal Kenya. We found that HRV occurs year-round in line with HRV prevalence reported among symptomatic individuals within the KHDSS (11%-23%) [35,36,41]. HRV was detected across

medRxiv preprint doi: https://doi.org/10.1101/2020.03.09.20033019.this version posted March 19, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

all age-groups, with the highest proportion in the <5 year-olds and the lowest proportion in older age groups(>=18 years), in agreement with previous findings [25,42,43]. A proportion of the HRV positive children (14%) identified another household member as having ARI-like symptoms, suggesting transmission at the household level that might contribute to transmission at school (or vice versa).

All HRV species were found in circulation throughout the year. HRV-A circulated widely (53%), more than -B (18%) and -C (29%), a contrast to previous reports where HRV-A and HRV-C co-dominated [35,36,41]. However, a similar occurrence of HRV species was reported in the first two years of aboriginal and non-aboriginal Australian children [44]. There was considerable HRV diversity, with almost a third of all known HRV genotypes detected. HRV infections comprised of single genotype occurrences, observed in a single sample/individual, as well as frequent genotypes affecting numerous pupils across several classes. Previous experimental studies have shown that different HRV types possess varying degrees of infectivity [45].

Numerous genotypes co-circulated in every school term, implying that no particular genotype predominates at any given time period. Previous studies have shown that contemporary HRV infections in a given population are characterised by numerous genotype-specific "mini-epidemics"[46] and that up to 30 genotypes circulate simultaneously at a given geographical area[47]. No frequent genotype was limited to one class, suggesting heterogeneous mixing and transmission within the school. However, four frequent genotypes and one variant of B70 were observed only in the lower primary, an indication of social clustering. Genotype recurrence in a subsequent school term was observed in nine genotypes. Although it is not clear whether the study design missed samples between two genotype occurrences, the infrequency of genotype recurrence is possibly a reflection of herd immunity to specific types within the school/local community or a

medRxiv preprint doi: https://doi.org/10.1101/2020.03.09.20033019.this version posted March 19, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

reflection of random introductions into the school/local community. Frequent genotypes in the school persisted for about a month on average. This is a shorter period than earlier observed across the KHDSS (a larger geographical scope) during an outpatient surveillance [41]. This is probably due to increased transmission (steered by high contact rates among school-going children) resulting in a shorter duration epidemic.

The younger age groups exhibited high rhinovirus diversity as they had more HRV re-infections. No individual was re-infected with the same genotype; further evidence of serotype-specific immunity to HRV lasting at least one year [48].

We demonstrate the occurrence of intra-genotype variants, which were either separate rhinovirus introductions or diversification of a single variant after introduction and as a result forming different transmission clusters. This observation highlights the benefit of sequence data over serology to study viral transmission dynamics.

An outpatient health facility located within the same location as the school was recruited into an ARI surveillance study [49] from December 2015 to November 2016, five months prior to our school study. This outpatient clinic is within a radius of 4 km from the school. Detailed analysis of molecular epidemiology of HRV for samples collected at this outpatient clinic was reported elsewhere [41] . Although not a primary objective of this study, we compared the diversity of HRV infections between the two study periods. We observed 12 common genotypes in the two studies: A13, A20, A28, A31, A46, A54, A78, A101, B42, C6, C11 and C19. However, only one genotype was frequent in both periods: A101 (**Supplementary figure 1**). Our comparison of HRV diversity between a school setting and clinical cases in a health facility within the same geographical location and in two consecutive seasons showed only one frequent genotype present in both studies. This is an indication that HRV diversity within a community varies widely over time, as

medRxiv preprint doi: https://doi.org/10.1101/2020.03.09.20033019.this version posted March 19, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

previously observed [50]. It is not definite what drives the exchange of common rhinovirus genotypes. The rapid turnover and co-existence of genotypes and variants might be determined by immunologically mediated selection processes or other nonselective epidemiological processes.

Our study had some limitations. First, the dichotomy in the number of samples collected weekly in lower primary versus upper primary posed a challenge when comparing the two groups. Second, weekly sampling of only symptomatic persons will likely have resulted in missed HRV infections, impairing the overview of HRV dynamics. In addition, the study failed to successfully amplify and sequence nearly 18% of HRV positive samples. Failure was not correlated with viral load and may have been caused by variability in primer-annealing sites resulting in mismatches. This may have resulted in missed genotypes or sub-variants.

This study provides improved knowledge of the diversity and temporal characteristics of HRV in a school setting, reinforcing the notion that schools are a focal point in understanding HRV transmission in the community. The effect of numerous individuals in close contact enabling HRV transmission is evident. In addition, we see that infections could be linked to transmission events occurring outside the school setting, i.e. household setting. The contemporary inclusion of different population structures (e.g. schools, households, health centres) in studying HRV dynamics will improve understanding of HRV epidemiology in communities. Future studies should focus on whole-genome sequencing to fully elucidate transmission clusters.

Data availability

The replication data and analysis scripts for this manuscript shall be made available from the Harvard Dataverse: (https://dataverse.harvard.edu/dataverse/vec). Some of the clinical dataset contains potentially identifying information on participants and is stored under restricted access.

medRxiv preprint doi: https://doi.org/10.1101/2020.03.09.20033019.this version posted March 19, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

Requests for access to the restricted dataset should be made to the Data Governance Committee (dgc@kemri-wellcome.org).

Competing interests

The authors declare no competing interests.

Acknowledgements

We thank all the study participants for their contribution of samples and data. We also thank the School teachers, School Management Board, Parent Teacher Association and County Education Management Committees for allowing us to conduct the study within their school. We are grateful to the field study team for participant recruitment and the laboratory staff of the KEMRI-Wellcome Trust Research Programme / Virus Epidemiology and Control research group. This paper is published with the permission of the Director of KEMRI.

Funding

This work was supported by the Wellcome Trust through a Wellcome Senior Investigator Award to the last author (#102975). This work was done in partial fulfilment of the first author's postgraduate diploma studentship. The studentship was supported through the DELTAS Africa Initiative [DEL-15-003]. The DELTAS Africa Initiative is an independent funding scheme of the African Academy of Sciences (AAS)'s Alliance for Accelerating Excellence in Science in Africa (AESA) and supported by the New Partnership for Africa's Development Planning and Coordinating Agency (NEPAD Agency) with funding from the Wellcome Trust [107769/Z/10/Z] and the UK government. The views expressed in this publication are those of the author(s) and not necessarily those of AAS, NEPAD Agency, Wellcome Trust or the UK government.

medRxiv preprint doi: https://doi.org/10.1101/2020.03.09.20033019.this version posted March 19, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY-NC-ND 4.0 International license .

REFERENCES

1. Wang K, Xi W, Yang D, et al. Rhinovirus is associated with severe adult community-acquired pneumonia in China. *J Thorac Dis*. 2017;9(11):4502-4511. doi:10.21037/jtd.2017.10.107
2. Peltola V, Waris M, Osterback R, Susi P, Hyypia T, Ruuskanen O. Clinical effects of rhinovirus infections. *J Clin Virol*. 2008;43(4):411-414. doi:10.1016/j.jcv.2008.08.014
3. Chen J, Hu P, Zhou T, et al. Epidemiology and clinical characteristics of acute respiratory tract infections among hospitalized infants and young children in Chengdu, West China, 2009–2014. *BMC Pediatr*. 2018;18. doi:10.1186/s12887-018-1203-y
4. Launes C, Armero G, Anton A, et al. Molecular epidemiology of severe respiratory disease by human rhinoviruses and enteroviruses at a tertiary paediatric hospital in Barcelona, Spain. *Clin Microbiol Infect*. 2015;21(8):799.e5-7. doi:10.1016/j.cmi.2015.04.021
5. Saraya T, Kimura H, Kurai D, Ishii H, Takizawa H. The molecular epidemiology of respiratory viruses associated with asthma attacks: A single-center observational study in Japan. *Med*. 2017;96(42):e8204. doi:10.1097/md.0000000000008204
6. Atkinson TP. Is asthma an infectious disease? New evidence. *Curr Allergy Asthma Rep*. 2013;13(6):702-709. doi:10.1007/s11882-013-0390-8
7. McLean HQ, Peterson SH, King JP, Meece JK, Belongia EA. School absenteeism among school-aged children with medically attended acute viral respiratory illness during three influenza seasons, 2012-2013 through 2014-2015. *Influ Other Respir Viruses*. 2017;11(3):220-229. doi:10.1111/irv.12440
8. Jacobs SE, Lamson DM, St George K, Walsh TJ. Human rhinoviruses. *Clin Microbiol Rev*. 2013;26(1):135-162. doi:10.1128/CMR.00077-12

medRxiv preprint doi: https://doi.org/10.1101/2020.03.09.20033019.this version posted March 19, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

9. Rotbart HA, Hayden FG. Picornavirus infections: a primer for the practitioner. *Arch Fam Med*. 2000;9(9):913-920.

10. Jennings LC, Dick EC. Transmission and control of rhinovirus colds. *Eur J Epidemiol*. 1987;3(4):327-335.

11. Winther B. Rhinovirus infections in the upper airway. *Proc Am Thorac Soc*. 2011;8(1):79-89. doi:10.1513/pats.201006-039RN

12. Hung IFN, Zhang AJ, To KKW, et al. Unexpectedly Higher Morbidity and Mortality of Hospitalized Elderly Patients Associated with Rhinovirus Compared with Influenza Virus Respiratory Tract Infection. *Int J Mol Sci*. 2017;18(2). doi:10.3390/ijms18020259

13. Denlinger LC, Sorkness RL, Lee WM, et al. Lower Airway Rhinovirus Burden and the Seasonal Risk of Asthma Exacerbation. *Am J Respir Crit Care Med*. 2011;184(9):1007-1014. doi:10.1164/rccm.201103-0585OC

14. Pierangeli A, Scagnolari C, Selvaggi C, et al. Rhinovirus frequently detected in elderly adults attending an emergency department. *J Med Virol*. 2011;83(11):2043-2047. doi:10.1002/jmv.22205

15. Lewis-Rogers N, Seger J, Adler FR. Human Rhinovirus Diversity and Evolution: How Strange the Change from Major to Minor. *J Virol*. 2017;91(7). doi:10.1128/JVI.01659-16

16. Simmonds P, Gorbalenya AE, Harvala H, et al. Recommendations for the nomenclature of enteroviruses and rhinoviruses. *Arch Virol*. 2020;165(3):793-797. doi:10.1007/s00705-019-04520-6

17. Wang L, Smith DL. Capsid structure and dynamics of a human rhinovirus probed by hydrogen exchange mass spectrometry. *Protein Sci*. 2005;14(6):1661-1672. doi:10.1110/ps.051390405

18. Conant RM, Hamparian V V. Rhinoviruses: basis for a numbering system. II. Serologic

medRxiv preprint doi: https://doi.org/10.1101/2020.03.09.20033019.this version posted March 19, 2020. The copyright holder for this preprint
(which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

characterization of prototype strains. *J Immunol*. 1968;100(1):114-119.

19. Cooney MK, Fox JP, Kenny GE. Antigenic groupings of 90 rhinovirus serotypes. *Infect Immun*. 1982;37(2):642-647.
20. Lau SKP, Yip CCY, Woo PCY, Yuen K-Y. Human rhinovirus C: a newly discovered human rhinovirus species. *Emerg Health Threats J*. 2010;3:e2. doi:10.3134/ehtj.10.002
21. Palmenberg AC, Gern JE. Classification and evolution of human rhinoviruses. *Methods Mol Biol*. 2015;1221:1-10. doi:10.1007/978-1-4939-1571-2_1
22. Simmonds P, McIntyre C, Savolainen-Kopra C, Tapparel C, Mackay IM, Hovi T. Proposals for the classification of human rhinovirus species C into genotypically assigned types. *J Gen Virol*. 2010;91(Pt 10):2409-2419. doi:10.1099/vir.0.023994-0
23. McIntyre CL, Knowles NJ, Simmonds P. Proposals for the classification of human rhinovirus species A, B and C into genotypically assigned types. *J Gen Virol*. 2013;94(Pt 8):1791-1806. doi:10.1099/vir.0.053686-0
24. Kiang D, Kalra I, Yagi S, et al. Assay for 5' noncoding region analysis of all human rhinovirus prototype strains. *J Clin Microbiol*. 2008;46(11):3736-3745. doi:10.1128/jcm.00674-08
25. Peltola V, Waris M, Osterback R, Susi P, Ruuskanen O, Hyypia T. Rhinovirus transmission within families with children: incidence of symptomatic and asymptomatic infections. *J Infect Dis*. 2008;197(3):382-389. doi:10.1086/525542
26. Kiti MC, Kinyanjui TM, Koech DC, Munywoki PK, Medley GF, Nokes DJ. Quantifying age-related rates of social contact using diaries in a rural coastal population of Kenya. *PLoS One*. 2014;9(8):e104786. doi:10.1371/journal.pone.0104786
27. Agoti CN, Munywoki PK, Phan MVT, et al. Transmission patterns and evolution of respiratory syncytial virus in a community outbreak identified by genomic analysis. *Virus*

medRxiv preprint doi: https://doi.org/10.1101/2020.03.09.20033019.this version posted March 19, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

Evol. 2017;3(1):vex006. doi:10.1093/ve/vex006

28. Munywoki PK, Koech DC, Agoti CN, et al. The source of respiratory syncytial virus infection in infants: a household cohort study in rural Kenya. *J Infect Dis*. 2014;209(11):1685-1692. doi:10.1093/infdis/jit828
29. Jefferson T, Del Mar C, Dooley L, et al. Physical interventions to interrupt or reduce the spread of respiratory viruses. *Cochrane database Syst Rev*. 2010;(1):CD006207. doi:10.1002/14651858.CD006207.pub3
30. Scott JAG, Bauni E, Moisi JC, et al. Profile: The Kilifi Health and Demographic Surveillance System (KHDSS). *Int J Epidemiol*. 2012;41(3):650-657. doi:10.1093/ije/dys062
31. Adema I, Kamau E, Nyiro JU, et al. Surveillance of respiratory viruses among children attending a primary school in rural coastal Kenya. *Wellcome Open Res*. 2020.[in press]
32. Berkley JA, Munywoki P, Ngama M, et al. Viral etiology of severe pneumonia among Kenyan infants and children. *Jama*. 2010;303(20):2051-2057. doi:10.1001/jama.2010.675
33. Kamau E, Agoti CN, Lewa CS, et al. Recent sequence variation in probe binding site affected detection of respiratory syncytial virus group B by real-time RT-PCR. *J Clin Virol*. 2017;88:21-25. doi:10.1016/j.jcv.2016.12.011
34. Hammitt LL, Kazungu S, Welch S, et al. Added Value of an Oropharyngeal Swab in Detection of Viruses in Children Hospitalized with Lower Respiratory Tract Infection. *J Clin Microbiol*. 2011;49(6):2318 LP - 2320. doi:10.1128/JCM.02605-10
35. Onyango CO, Welch SR, Munywoki PK, et al. Molecular epidemiology of human rhinovirus infections in Kilifi, coastal Kenya. *J Med Virol*. 2012;84(5):823-831. doi:10.1002/jmv.23251
36. Kamau E, Onyango CO, Otieno GP, et al. An Intensive, Active Surveillance Reveals

medRxiv preprint doi: https://doi.org/10.1101/2020.03.09.20033019.this version posted March 19, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

Continuous Invasion and High Diversity of Rhinovirus in Households. *J Infect Dis*. 2019;219(7):1049-1057. doi:10.1093/infdis/jiy621

37. Yamada KD, Tomii K, Katoh K. Application of the MAFFT sequence alignment program to large data-reexamination of the usefulness of chained guide trees. *Bioinformatics*. 2016;32(21):3246-3251. doi:10.1093/bioinformatics/btw412

38. Nguyen LT. IQ-TREE: A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies. 2015;32(1):268-274. doi:10.1093/molbev/msu300

39. Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol Biol Evol*. 2016;33(7):1870-1874. doi:10.1093/molbev/msw054

40. Kiyota N, Kobayashi M, Tsukagoshi H, et al. Genetic analysis of human rhinovirus species A to C detected in patients with acute respiratory infection in Kumamoto prefecture, Japan 2011-2012. *Infect Genet Evol*. 2014;21:90-102. doi:10.1016/j.meegid.2013.10.024

41. Morobe JM, Nyiro JU, Brand S, et al. Human rhinovirus spatial-temporal epidemiology in rural coastal Kenya, 2015-2016, observed through outpatient surveillance. *Wellcome open Res*. 2018;3:128. doi:10.12688/wellcomeopenres.14836.2

42. Kieninger E, Fuchs O, Latzin P, Frey U, Regamey N. Rhinovirus infections in infancy and early childhood. *Eur Respir J*. 2013;41(2):443 LP - 452. doi:10.1183/09031936.00203511

43. Baillie VL, Moore DP, Mathunjwa A, Morailane P, Simoes EAF, Madhi SA. Molecular Subtyping of Human Rhinovirus in Children from Three Sub-Saharan African Countries. *J Clin Microbiol*. 2019;57(9). doi:10.1128/JCM.00723-19

44. Annamalay AA, Khoo S-K, Jacoby P, et al. Prevalence of and risk factors for human rhinovirus infection in healthy aboriginal and non-aboriginal Western Australian children.

medRxiv preprint doi: https://doi.org/10.1101/2020.03.09.20033019.this version posted March 19, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

Pediatr Infect Dis J. 2012;31(7):673-679. doi:10.1097/INF.0b013e318256ffc6

45. MONTO AS, CAVALLARO JJ. THE TECUMSEH STUDY OF RESPIRATORY ILLNESSIV. PREVALENCE OF RHINOVIRUS SEROTYPES, 1966–196912. *Am J Epidemiol*. 1972;96(5):352-360. doi:10.1093/oxfordjournals.aje.a121466
46. Martin ET, Kuypers J, Chu HY, et al. Heterotypic Infection and Spread of Rhinovirus A, B, and C among Childcare Attendees. *J Infect Dis*. 2018;218(6):848-855. doi:10.1093/infdis/jiy232
47. Mackay IM, Lambert SB, Faux CE, et al. Community-Wide, Contemporaneous Circulation of a Broad Spectrum of Human Rhinoviruses in Healthy Australian Preschool-Aged Children During a 12-Month Period. *J Infect Dis*. 2012;207(9):1433-1441. doi:10.1093/infdis/jis476
48. Barclay WS, al-Nakib W, Higgins PG, Tyrrell DA. The time course of the humoral immune response to rhinovirus infection. *Epidemiol Infect*. 1989;103(3):659-669. doi:10.1017/s095026880003106x
49. Nyiro JU, Munywoki P, Kamau E, et al. Surveillance of respiratory viruses in the outpatient setting in rural coastal Kenya: baseline epidemiological observations. *Wellcome open Res*. 2018;3:89. doi:10.12688/wellcomeopenres.14662.1
50. Monto AS. Epidemiology of viral respiratory infections. *Am J Med*. 2002;112 Suppl:4S-12S. doi:10.1016/s0002-9343(01)01058-0

medRxiv preprint doi: https://doi.org/10.1101/2020.03.09.20033019.this version posted March 19, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

TABLES AND FIGURES

Table legend

Table 1. Baseline characteristics of the HRV positive cases at a rural Kenyan school sampled throughout 3 terms from May 2017 to April 2018.

Table 2. Human rhinovirus detection and genotyping summary among individuals.

medRxiv preprint doi: https://doi.org/10.1101/2020.03.09.20033019.this version posted March 19, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

Table 1. Baseline characteristics of the HRV positive cases at a rural Kenyan school sampled throughout 3 terms from May 2017 to April 2018.

Characteristic	Categories	No. Pos	% Pos	No. Neg	% Neg	Total
Age (pupils)	<=5yrs	78	22.4	270	77.6	348
	6-10yrs	137	17.6	641	82.4	778
	11-17yrs	82	12.4	578	87.6	660
	>=18yrs	2	5.9	32	94.1	34
	Unspecified	3	42.9	4	57.1	7
Age (teachers)	>=18yrs	5	15.6	27	84.4	32
	Total	307	16.5	1552	83.5	1859
Gender	Male	169	18.8	731	81.2	900
	Female	138	14.4	821	85.6	959
Symptoms in the last two weeks						
Cough	Yes	226	16.6	1137	83.4	1363
	No	81	16.4	413	83.6	494
Nasal Discharge	Yes	278	17.0	1357	83.0	1635
	No	29	13.1	193	86.9	222
Sore throat	Yes	62	16.2	321	83.8	383
	No	245	16.6	1227	83.4	1472
Household (HH) members						
Other persons in HH with symptoms of ARI?	Yes	43	14.2	260	85.8	303
	No	215	17.4	1024	82.7	1239
	Don't know	49	15.5	268	84.5	317
Are they in school? (n=43)	Yes	26	16.1	136	84.0	162
	No	17	12.1	124	87.9	141

medRxiv preprint doi: https://doi.org/10.1101/2020.03.09.20033019.this version posted March 19, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

	Unspecified	264	17.0	1292	83.0	1556

No. Pos: Number positive

% Pos: Percent positive

No. Neg: Number negative

% Neg: Percent negative

medRxiv preprint doi: https://doi.org/10.1101/2020.03.09.20033019.this version posted March 19, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

Table 2. Human rhinovirus detection and genotyping summary among individuals.

Distribution of HRV among individuals	**Frequency of HRV positive samples/ person**	**Number of individuals**	**Percentage %**
	One	102	62.2
	Two	31	18.9
	Three	13	7.9
	Four	5	3.0
	Five	3	1.8
	Six	4	2.4
	Seven	3	1.8
	Eight	3	1.8
	Total	164	100
Distribution of sequenced samples among individuals	**Frequency of sequenced samples/ person**	**Number of individuals**	**Percentage %**
	One	96	66.7
	Two	23	16.0
	Three	9	6.3
	Four	5	3.5
	Five	5	3.5
	Six	4	2.8
	Seven	1	0.7
	Eight	1	0.7
	Total	144	100
Diversity of HRV	**Number of unique types/ person**	**Number of individuals**	**Percentage %**
	One	98	68.1
	Two	22	15.3

medRxiv preprint doi: https://doi.org/10.1101/2020.03.09.20033019.this version posted March 19, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

	Three	12	8.3
	Four	9	6.3
	Five	3	2.1
	Total	144	100

medRxiv preprint doi: https://doi.org/10.1101/2020.03.09.20033019.this version posted March 19, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

Figure legend

Figure 1. Patterns of HRV infections within the school over one year. (A). Month by month HRV proportion with respective 95% confidence intervals and number of samples tested. (B). Class-specific HRV proportion with respective 95% confidence intervals. The two bars furthest to the right are aggregated proportions of lower primary and upper primary groups.

Figure 2. Phylogenetic analysis and genotype assignment of generated HRV sequences. Species-specific maximum likelihood trees of (A) HRV-A, (B) HRV-B and (C) HRV-C. The tip shapes are colored by the school class of the individual. The black tips represent the prototype sequence of respective strain. (D) A circular bar plot showing the frequencies of HRV genotypes identified. The bars are colored by HRV species and the tips are labeled by: HRV-type, frequency.

Figure 3. The temporal occurrence and infection patterns of HRV. (A) The temporal occurrence of all HRV genotypes across the one-year study period. (B) Individual infection patterns across the one-year period. The individuals are ordered by date of first HRV infection. (C) Number of days to a subsequent HRV case for individuals who were re-infected during the study period. All individual first infections are dated day 0. The individuals are ordered by number of HRV positive samples, with the individual with most positive samples appearing at the bottom.

Figure 4. Distribution of genotypes across the school. (A) Heatmap showing the distribution of frequent genotypes across the school classes. The intensity of the color correlates to frequency of samples. Color intensity has been scaled to correct for sampling bias between the lower primary and upper primary classes. (B) Distribution of frequent genotypes between the upper and lower primary groups. The sizes of the circles correlate to number of samples and the color to the respective group: either lower or upper primary.

medRxiv preprint doi: https://doi.org/10.1101/2020.03.09.20033019.this version posted March 19, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

Figure 5. Genetic diversity of HRV genotypes with a frequency of more than ten. From top left A2, A28, A36, B48-like, B70, C_pat19, C3 and C13 (bottom right). Nucleotide substitutions are demonstrated by a colored bar. A substitution to an "A" is indicated by green, "T" by red, "G" by indigo and "C" by blue bars. The sequences are ordered by date of sample collection with the genotype's index sequence at the bottom, and acting as a reference.

medRxiv preprint doi: https://doi.org/10.1101/2020.03.09.20033019.this version posted March 19, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

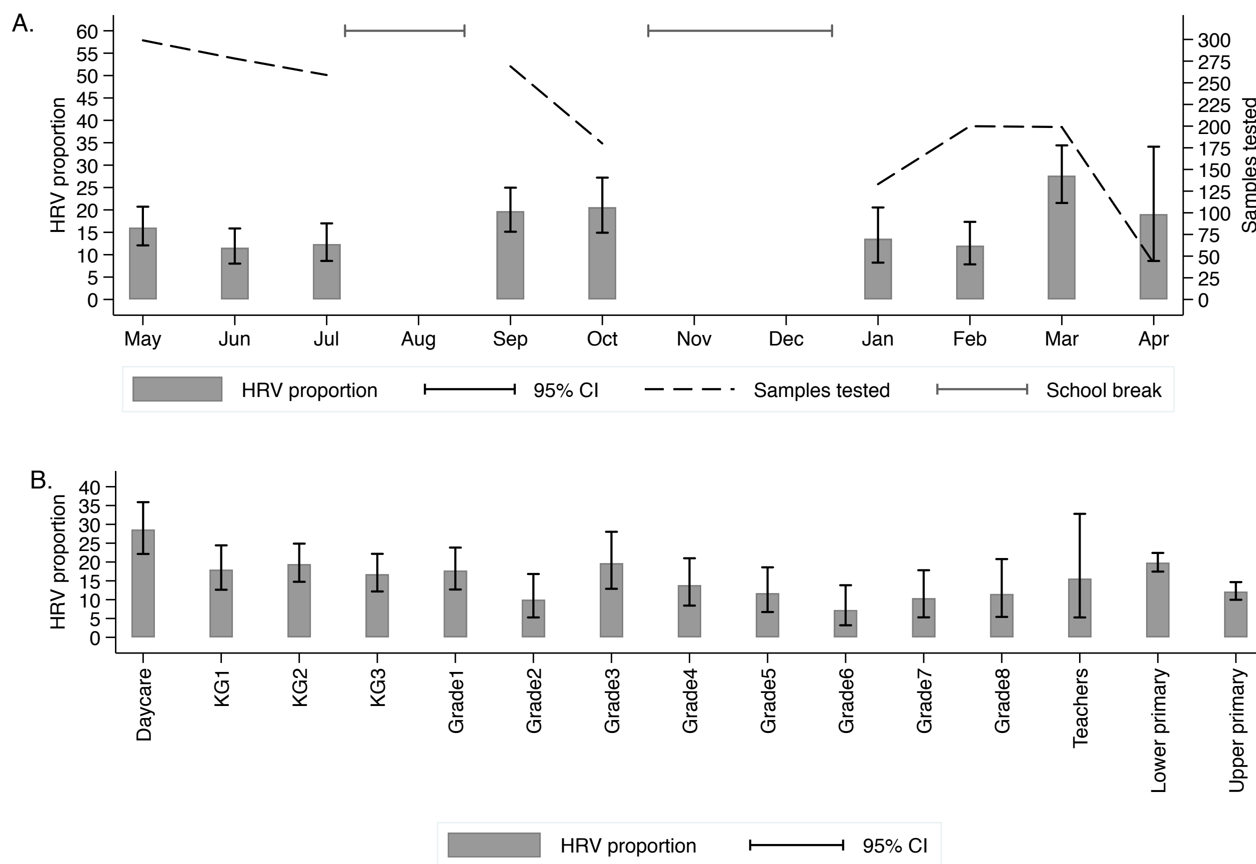


Figure 1. Patterns of HRV infections within the school over one year. (A). Month by month HRV proportion with respective 95% confidence intervals and number of samples tested. (B). Class-specific HRV proportion with respective 95% confidence intervals. The two bars furthest to the right are aggregated proportions of lower primary and upper primary groups.

medRxiv preprint doi: https://doi.org/10.1101/2020.03.09.20033019.this version posted March 19, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

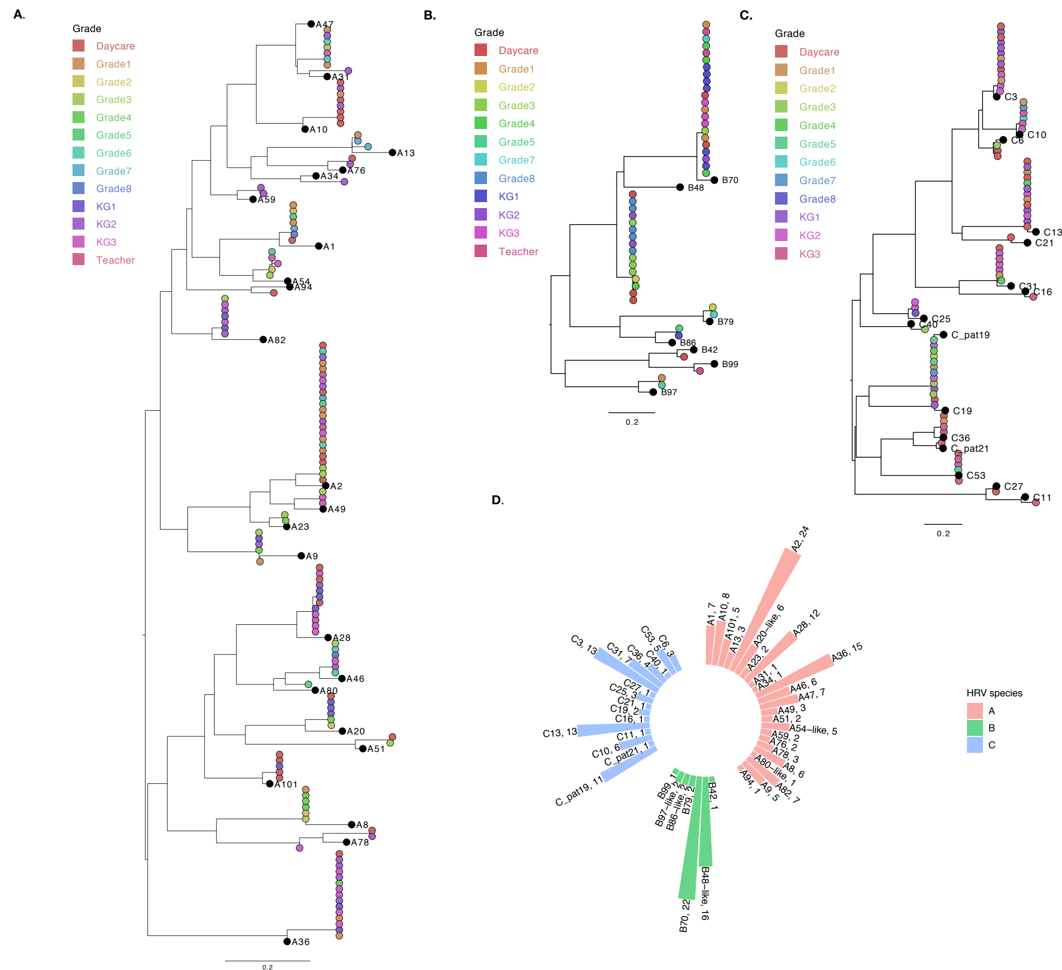


Figure 2. Phylogenetic analysis and genotype assignment of generated HRV sequences. Species-specific maximum likelihood trees of (A) HRV-A, (B) HRV-B and (C) HRV-C. The tip shapes are colored by the school class of the individual. The black tips represent the prototype sequence of respective strain. (D) A circular bar plot showing the frequencies of HRV genotypes identified. The bars are colored by HRV species and the tips are labeled by: HRV-type, frequency.

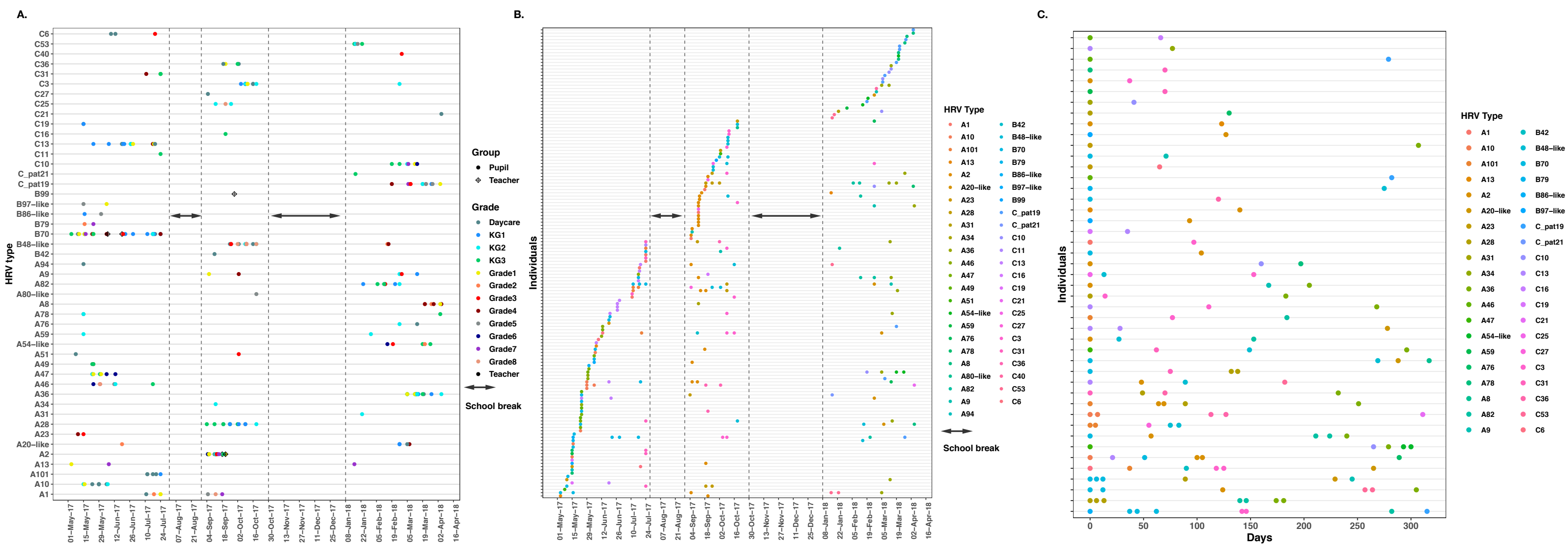
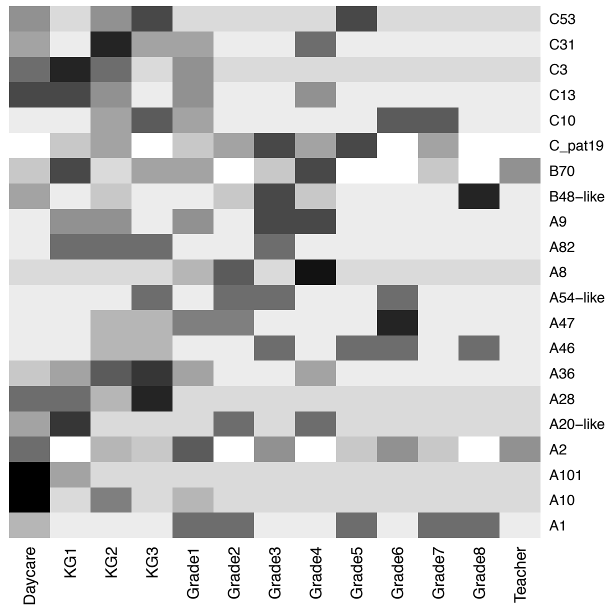


Figure 3. The temporal occurrence and infection patterns of HRV. (A) The temporal occurrence of all HRV genotypes across the one-year study period. (B) Individual infection patterns across the one-year period. The individuals are ordered by date of first HRV infection. (C) Number of days to a subsequent HRV case for individuals who were re-infected during the study period. All individual first infections are dated day 0. The individuals are ordered by number of HRV positive samples, with the individual with most positive samples appearing at the bottom.

A.



B.

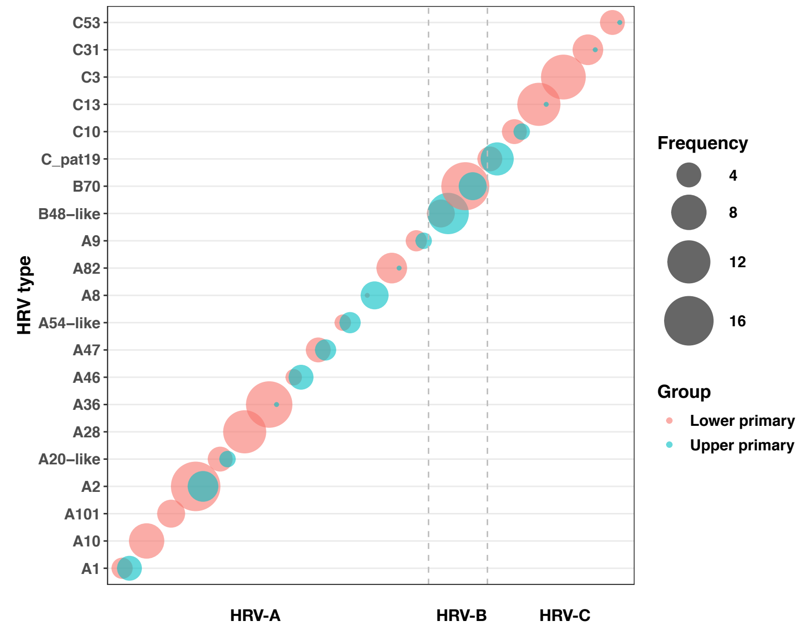


Figure 4. Distribution of genotypes across the school. (A) Heatmap showing the distribution of frequent genotypes across the school classes. The intensity of the color correlates to frequency of samples. Color intensity has been scaled to correct for sampling bias between the lower primary and upper primary classes. (B) Distribution of frequent genotypes between the upper and lower primary groups. The sizes of the circles correlate to number of samples and the color to the respective group: either lower or upper primary.

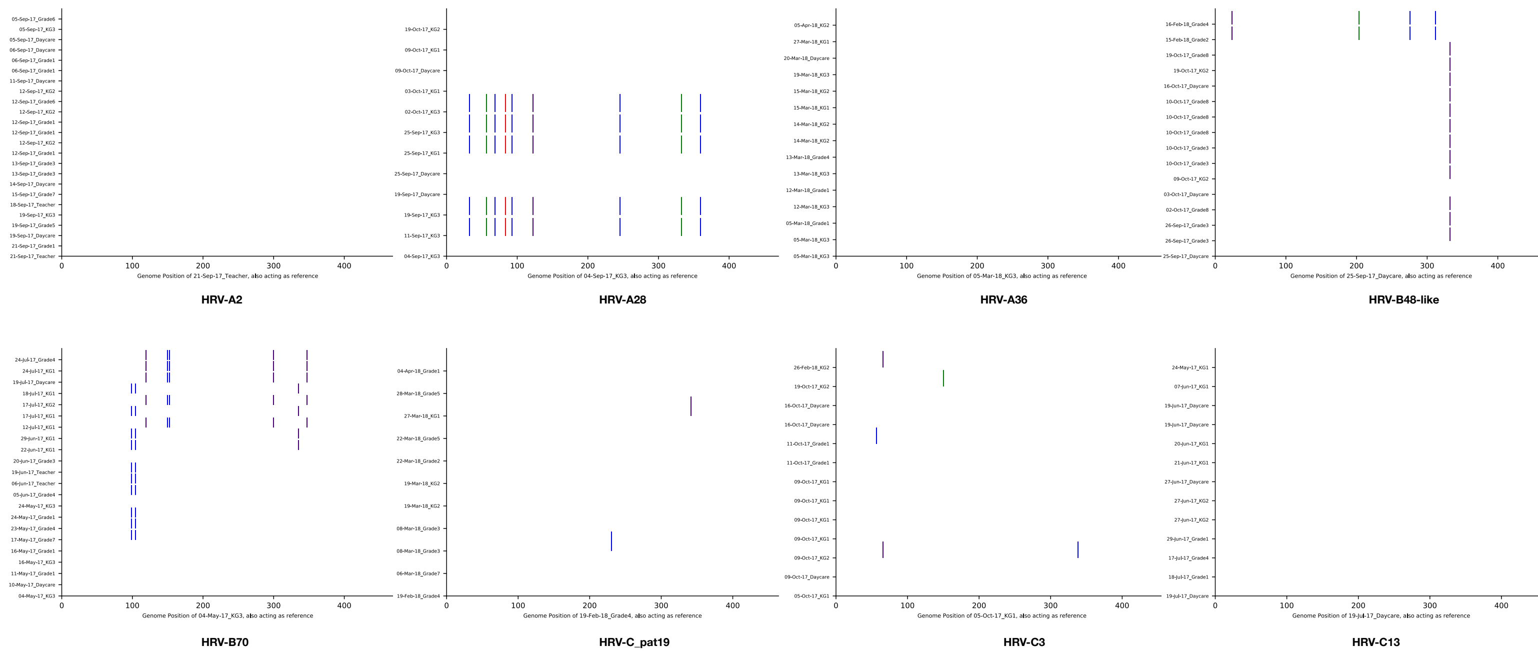


Figure 5. Genetic diversity of HRV genotypes with a frequency of more than ten. From top left A2, A28, A36, B48-like, B70, C_pat19, C3 and C13 (bottom right). Nucleotide substitutions are demonstrated by a colored bar. A substitution to an "A" is indicated by green, "T" by red, "G" by indigo and "C" by blue bars. The sequences are ordered by date of sample collection with the genotype's index sequence at the bottom, and acting as a reference.

medRxiv preprint doi: https://doi.org/10.1101/2020.03.09.20033019.this version posted March 19, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

Supplementary Material

Supplementary Table 1: Limits of intra-type pairwise genetic distances of Kilifi school sequences to HRV prototype strains

Type	Accession number of prototype strain	Number of sequences	Upper limit p-distance
A1	D002390.1	7	0.093
A10	DQ4734980.1	8	0.071
A101	GQ4150510.1	5	0.044
A13	FJ4451160.1	3	0.099
A2	X023160.1	24	0.102
A20-like *	FJ4451200.1	6	0.118
A23	DQ4734970.1	2	0.074
A28	DQ4735080.1	12	0.102
A31	FJ4451260.1	1	0.104
A34	DQ4735010.1	1	0.093
A36	DQ4735050.1	15	0.091
A46	DQ4735060.1	6	0.071
A47	FJ4451330.1	7	0.085
A49	DQ4734960.1	3	0.102
A51	FJ4451360.1	2	0.088
A54-like *	FJ4451380.1	5	0.107
A59	DQ4735000.1	2	0.088
A76	DQ4735020.1	2	0.082
A78	EF1734180.1	3	0.102
A8	FJ4451130.1	6	0.085
A80-like*	FJ4451560.1	1	0.137
A82 *	DQ4735090.1	7	0.113
A9	FJ4451770.1	5	0.077
A94	EF1734190.1	1	0.102
B42	FJ4451300.1	1	0.073
B48-like *	DQ4734880.1	16	0.154
B70	DQ4734890.1	22	0.09
B79	FJ4451550.1	2	0.051
B86-like *	FJ4451640.1	2	0.121
B97-like *	FJ4451720.1	2	0.118
B99	FJ4451740.1	1	0.076
C_pat19	FJ5980960.1	11	0.047
C_pat21	FJ6157370.1	1	0.061
C10	GQ3237740.1	6	0.05
C11	EU8409520.1	1	0.087
C13	EU0817950.1	13	0.041
C16	EU0818080.1	1	0.047
C19	EU6978500.1	2	0.055

medRxiv preprint doi: https://doi.org/10.1101/2020.03.09.20033019.this version posted March 19, 2020. The copyright holder for this preprint
(which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

C21	EU7523770.1	1	0.073
C25	EU7524270.1	3	0.105
C27	GQ2231220.1	1	0.026
C3	EF1860770.1	13	0.052
C31	GU2943800.1	7	0.079
C36	EF0772560.1	4	0.093
C40	EU0818020.1	1	0.064
C53	MF7753670.1	5	0.006
C6	EF5823870.1	3	0.061

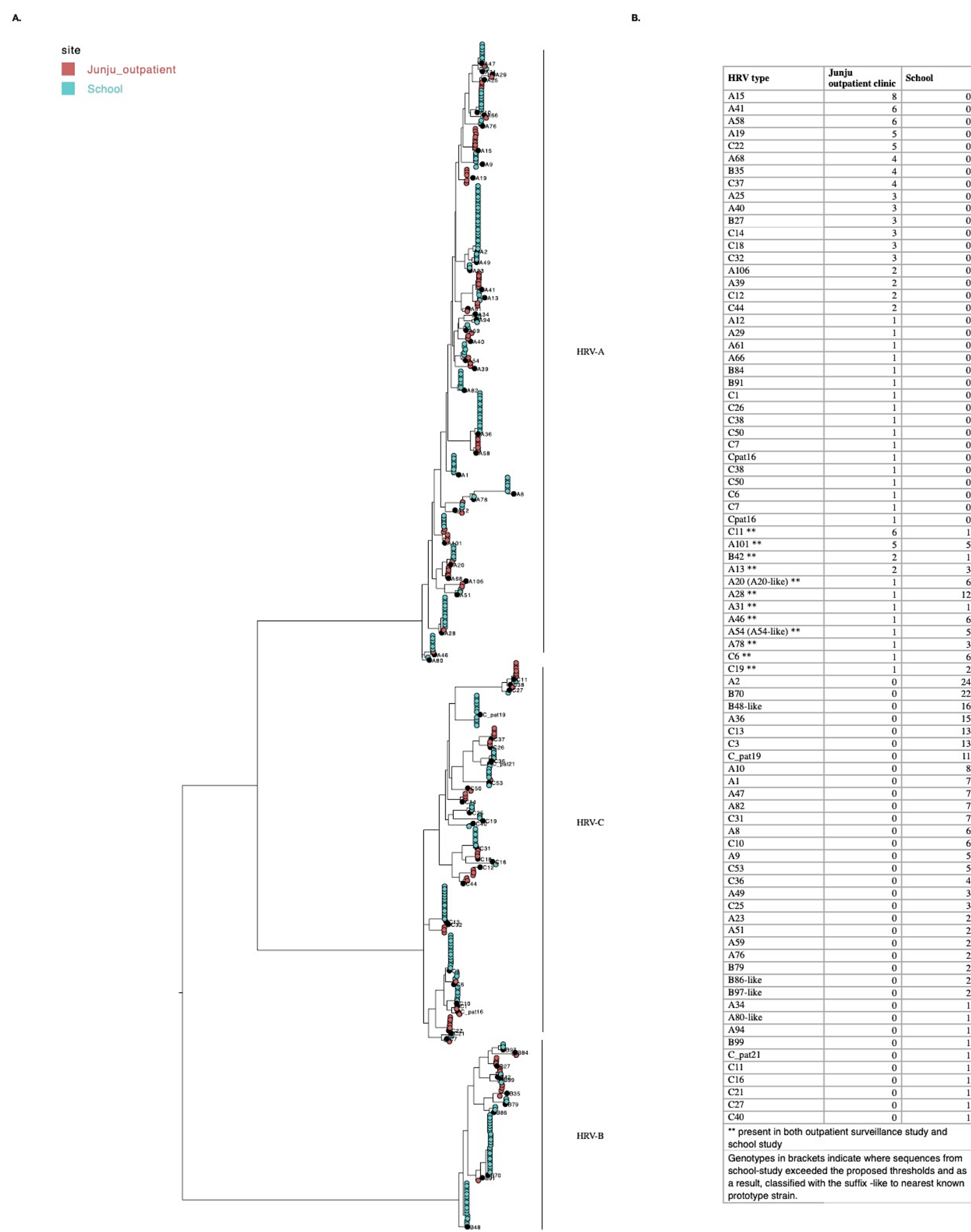
* Do not conform to proposed VP4/2 thresholds. This has been documented for genotypes A78 (intra-clade upper limit of 0.153), A82 (intra-clade upper limit of 0.135) and A20 (intra-clade upper limit of 0.119) [23].

medRxiv preprint doi: https://doi.org/10.1101/2020.03.09.20033019.this version posted March 19, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

Supplementary Figure legend

Supplementary Figure 1: Genotype distribution between the school and the Junju outpatient clinic. (A). Phylogenetic analysis of sequences from the two studies. The tips are colored by study site. (B). A table with genotype frequencies from both studies. Only 12 genotypes were present in both studies.

medRxiv preprint doi: https://doi.org/10.1101/2020.03.09.20033019.this version posted March 19, 2020. The copyright holder for this preprint
(which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .



HRV type	Junju outpatient clinic	School
A15	8	0
A41	6	0
A58	6	0
A19	5	0
C22	5	0
A68	4	0
B35	4	0
C37	4	0
A25	3	0
A40	3	0
B27	3	0
C14	3	0
C18	3	0
C32	3	0
A106	2	0
A39	2	0
C12	2	0
C44	2	0
A12	1	0
A29	1	0
A61	1	0
A66	1	0
B84	1	0
B91	1	0
C1	1	0
C26	1	0
C38	1	0
C50	1	0
C7	1	0
Cpat16	1	0
C38	1	0
C50	1	0
C6	1	0
C7	1	0
Cpat16	1	0
C11 **	6	1
A101 **	5	5
B42 **	2	1
A13 **	2	3
A20 (A20-like) **	1	6
A28 **	1	12
A31 **	1	1
A46 **	1	6
A54 (A54-like) **	1	5
A78 **	1	3
C6 **	1	6
C19 **	1	2
A2	0	24
B70	0	22
B48-like	0	16
A36	0	15
C13	0	13
C3	0	13
C_pat19	0	11
A10	0	8
A1	0	7
A47	0	7
A82	0	7
C31	0	7
A8	0	6
C10	0	6
A9	0	5
C53	0	5
C36	0	4
A49	0	3
C25	0	3
A23	0	2
A51	0	2
A59	0	2
A76	0	2
B79	0	2
B86-like	0	2
B97-like	0	2
A34	0	1
A80-like	0	1
A94	0	1
B99	0	1
C_pat21	0	1
C11	0	1
C16	0	1
C21	0	1
C27	0	1
C40	0	1

** present in both outpatient surveillance study and school study

Genotypes in brackets indicate where sequences from school-study exceeded the proposed thresholds and as a result, classified with the suffix -like to nearest known prototype strain.

Supplementary Figure 1: Genotype distribution between the school and the Junju outpatient clinic. (A). Phylogenetic analysis of sequences from the two studies. The tips are colored by study site. (B). A table with genotype frequencies from both studies. Only 12 genotypes were present in both studies.