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1 **Absence of back to school peaks in human rhinovirus detections and**  
2 **respiratory symptoms in a cohort of children with asthma**  
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## Abstract

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Much of what is known about the seasonality of human rhinovirus (hRV) infections has been learned from the study of acute asthma exacerbations presenting to emergency care, including those among children at the start of the school term. Much less is known about the patterns of hRVs in the community. In this study, viruses and day-to-day symptoms of asthma and colds were monitored twice weekly in 67 children with asthma aged 5-12 years, over a 15 month period in Sydney, Australia.

Overall hRV was detected in 314/1232 (25.5%) of nasal wash samples and 142/1231 (11.5%) of exhaled breath samples, and 123 and 24 respectively were sequenced for hRV genotype. HRVs were detected with similar prevalence rate throughout the year, including no peak in hRV prevalence following return to school. No peaks were seen in asthma and cold symptoms using twice-weekly diary records. However, over the same period in children of the same age, there were peaks in asthma emergency visits both at a large local hospital and in state-wide hospitalisations, following both return to school (February) and in late autumn (May).

This study suggests that hRV infections are common throughout the year among children, and differences in virus prevalence alone may not account for peaks in asthma symptoms.

## Keywords

Human rhinovirus, respiratory viruses, asthma, hospital admission for asthma, emergency department presentations for asthma, asthma exacerbations

## 1 **Introduction**

2 Human rhinovirus (hRV) infections are the most frequent cause of upper respiratory tract  
3 infections (URTI, or 'colds') in both children and adults. They have been strongly associated  
4 with acute exacerbations of asthma requiring medical intervention (Busse et al., 2010). In  
5 longitudinal studies in the northern and southern hemispheres, asthma exacerbations in  
6 children show a strong seasonal peak in late summer-autumn, following return to school  
7 (Johnston et al., 2005; Lincoln et al., 2006; Sears and Johnston 2007). Samples collected  
8 from children during these exacerbations are frequently hRV positive (Johnston et al. 2005).  
9 HRV infections are also significantly associated with day-to-day respiratory symptoms in  
10 children (Tovey et al., 2014).

11 There are greater than 168 serotypes and genotypes of hRVs, currently defined on a  
12 molecular basis as three species: hRV-A, -B, -C (Simmonds et al., 2010; Miller and Mackay  
13 2013). There is as yet no consensus as to the seasonality of the three species (Olenec et al.,  
14 2010; Arakawa et al., 2012; Linder et al., 2013).

15 There are a number of challenges in determining the role of hRV in day-to-day asthma  
16 symptoms and asthma exacerbations. Much of our current understanding of the seasonal  
17 prevalence of circulating hRVs is based on studies concerned with more severe infections or  
18 those with a greater impact on asthma. These include data from acute hospital admissions  
19 (Druce et al., 2005), prospective sampling in periods of increased symptomatology(Olenec et  
20 al. 2010) and performing sampling on children when they are symptomatic (MacKay et al.,  
21 2013). In order to understand patterns of hRV circulating day-to-day in a community of  
22 children with asthma, samples should be collected at frequent intervals over a prolonged time  
23 course.

1 In a recent study, we analyzed nasal wash and exhaled breath samples were analyzed as part  
2 of a longitudinal study of children with asthma –SAVE (The Study of Asthma, Viruses and  
3 Environment) (Tovey et al. 2014). Sixty-seven children with asthma self-sampled twice a  
4 week for an average of 10 weeks. The current paper aimed to determine whether the back to  
5 school peaks in asthma [in the community](#) were reflected in the day to day hRV detections and  
6 asthma symptoms of the children enrolled in the SAVE cohort.

7

## 1 **Materials and Methods**

### 2 **Study design and participants**

3 SAVE was a longitudinal cohort study that involving 67 children aged between 5 and 12  
4 years with doctor diagnosed asthma; they were recruited from Sydney Children's Hospital  
5 (SCH), Randwick, [New South Wales \(NSW\)](#), Australia, either from the asthma outpatient  
6 clinic or from amongst hospital records of children with an ED attendance with a discharge  
7 diagnosis of asthma in the previous 12 months. The full methods and outcomes of day to day  
8 fluctuation of virus detection and respiratory symptoms have been reported (Tovey et al.  
9 2014).

10 Written informed consent was obtained from both parent and child. This study was approved  
11 by the Sydney Children's Network Human Ethics Committee (HREC Approval: 11/CHW/90,  
12 SSA Approval: SSA/11/SCHN/168).

### 13 **Sampling methods**

14 Details of sample collection, lung function measurement and questionnaires have been  
15 described (Tovey et al. 2014). Briefly, participants self-collected their nasal mucus and  
16 exhaled breath twice a week for up to 10 weeks. Twice each week, participants completed  
17 two diary cards with questions relating to asthma (Marks et al., 2010) and common cold  
18 symptoms. For asthma symptoms, a 4-point [severity](#) scale ~~of severity~~ was used to separately  
19 ~~measure-record~~ 'cough and phlegm' and 'wheeze and chest tightness' symptoms -since the  
20 diary was previously completed (yes/no). Cold symptoms were also reported twice-weekly  
21 based on the Common Cold Questionnaire (Powell et al., 2008), using a 4 point [severity](#) scale  
22 ~~of severity~~ to measure nine items typically associated with URTIs (fevers, chills, muscle  
23 pains, watery eyes, runny nose, sneezing, sore throat, cough and chest pain) over the previous  
24 3 days.

**Commented [HKR2]:** This is so the reader will understand why we report NSW school terms.

1 Sampling was conducted from October 2011 to December 2012. The [New South Wales](#)  
2 [\(NSW\)](#) Government school terms in 2012 were term 1: 27 January-5 April, term 2: 23 April-  
3 29 June, term 3: 16 July-21 September, term 4: 8 October-21 December, i.e. with four 10-  
4 week terms separated by 2-week vacations, and with a 6-week summer vacation in  
5 December/January. “Seasons” in Australia describe summer (December, January, February),  
6 autumn (March, April, May), winter (June, July, August) and spring (September, October,  
7 November).

### 8 **Molecular detection of respiratory viruses**

9 Methods used for the detection and identification of viruses have been described previously  
10 (Tovey et al., 2014).

### 11 **Contemporaneous meteorological data and asthma-related health care utilization**

12 The Bureau of Meteorology (BOM) provided meteorological data. Fortnightly average air  
13 temperatures were calculated from all temperature data collected for that fortnight at the  
14 Sydney Airport weather station (the closest station to SCH, [~4 miles](#)). Average percent  
15 relative humidity (RH%) values at the Sydney Airport weather station were calculated using  
16 the average air and dew point temperatures for each fortnight using the moisture equations  
17 provided by BOM.

18 Data on ED presentations for asthma for children 5-12 years old in the same geographic  
19 region were obtained from the SCH ED Data Collection. State-wide data on hospital  
20 admissions for asthma amongst children aged 5-12 years old were obtained from the  
21 Admitted Patient Data Collection (APDC) which covers the State of NSW (population 7.5  
22 million).

1 **Data analyses**

2 The proportion of virus positive nasal samples at different times over the course of the 15  
3 months of the study was calculated for: (i) all hRV positive samples, (ii) for each of the three  
4 species of hRV and (iii) for the non-hRVs, by dividing the number of positive samples by the  
5 total number tested, for each 4 week period of sampling. Confidence intervals were  
6 calculated using Fleiss' method (Fleiss 1981).

7 The relative abundance of the total hRV and three species of hRV in nasal wash samples  
8 collected in school vacations and during school terms was compared by examining the  
9 occurrence in samples collected in the sum of all four school vacations, with the sum in the  
10 samples collected during the four terms. Chi-square test was used to determine significant  
11 differences between hRV species detected. A p value of  $<0.05$  was considered to indicate a  
12 statistically significant association.

13 The occurrence of asthma symptoms over time was plotted as the fortnightly averages of the  
14 continuous scores for the two asthma symptoms (cough and phlegm / wheeze and chest  
15 tightness) as reported by questionnaire. Fortnightly averages of the total CCQ scores were  
16 plotted using a similar approach.

17 To determine the association between the rates of hRV positivity in our cohort and the state-  
18 wide hospital admission rate for asthma, a locally weighted non-parametric smoothing  
19 (LOESS) filter was applied to the series of fortnightly hospital admission rates. This was  
20 implemented using a Generalised Additive Model (GAM) using a log link with a Poisson  
21 distribution (PROC GAM, SAS 9.3, SAS Institute, Cary, NC). Partial autocorrelation  
22 function (PACF) plots were examined for models with time smoothing functions using  
23 degrees of freedom ranging from 1 to 8. The model with the least degrees of freedom that  
24 minimised the PACF was selected. The residuals from the selected model were used in the



1 subsequent stages of the analysis. In the second stage a regression model was fitted with the  
2 residuals from the smoothed series of hospital attendance rates as the dependent variable and  
3 the total samples that were positive for hRV as the independent variable.

4 The diversity of the different hRV strains over time was examined by combining the results  
5 for the hRV-positive nasal wash and exhaled breath samples and tabulating the number of  
6 times each type was first reported in a participant, over the collective school terms versus  
7 collective school vacations.

8

## 1 Results

### 2 Participants' characteristics

3 Sixty seven participants were enrolled in the study and 62 (94%) completed at least five  
4 weeks of data collection (Tovey et al. 2014). Participant characteristics shown in Table 1  
5 indicate that most children had well-controlled asthma at entry, based on the validated  
6 Children's Asthma Control Test (Liu et al., 2010).

### 7 Detection of virus

8 In total, 1232 nasal wash samples (Fig 1A) and 1231 exhaled breath samples were collected  
9 between October 2011 and December 2012. This 15-month period was the amount of time  
10 taken for recruitment, sampling and followup of all 67 participants. The rate of sample  
11 collection varied, with 5.0% of total samples collected in the last quarter of 2011, and 40.2 %,  
12 21.7%, 22.1% and 10.9% collected in the four quarters of 2012 (Tovey et al. 2014). There  
13 were between 6 and 31 participants sampling each month (see online supplement Figure S1).

14 Overall 314 (314/1232, 25.5%) nasal and 142 (142/1231, 11.5%) breath samples were  
15 positive for hRV over the 15 months. Of the 456 samples positive for hRV, 255/456 (55.9%),  
16 comprising 231/314 (73.6%) of positive nasal wash samples and 24/142 (17%) of exhaled  
17 breath samples, were able to be sequenced in the VP4-VP2 region of the hRV genome.

18 The rate of detection in nasal wash samples of total hRVs, the three hRV species, and non-  
19 hRVs, along with the numbers of samples collected, in each 4-week period is in Fig 1. HRVs  
20 occurred throughout the year. We observed no peak in hRV following return to school in  
21 early February (Figure 1A). The pattern of occurrence observed for hRV-A was similar to  
22 total hRV (Figure 1B) whereas the occurrence of hRV-B was confined to a period from  
23 January 2012-July 2012 (Figure 1C). HRV-C occurred throughout the year (Figure 1D). The

Commented [HKR3]: It took me a few attempts to understand this.

1 proportions of positive total hRV and hRV species in nasal wash samples collected in  
2 vacations and school terms were compared, and there was no significant difference between  
3 either period for total hRV ( $p=0.363$ ) or the species collectively ( $p=0.665$ ).

4 All non-hRV viruses tested for were detected (influenza A, influenza B, parainfluenza types  
5 1, 2, and 3, respiratory syncytial virus (RSV), and human metapneumovirus). However, very  
6 low numbers were observed (Figure 1E); only 28/1232 (2.3%) of the nasal wash samples and  
7 19/1231 (1.5%) of the exhaled samples were positive for non-hRV viruses, so these were not  
8 analysed further in this paper.

#### 9 **Fluctuation of respiratory symptoms in the SAVE cohort**

10 A total of 1206 symptom diary records were collected. Asthma symptoms ('wheeze and chest  
11 tightness' and 'cough and phlegm') fluctuated throughout the year in the SAVE population,  
12 but we observed no peaks of reported symptoms over the 15 months (Figure 2A). In winter  
13 and spring, children reported more 'cough and phlegm' than 'wheeze and chest tightness'.  
14 Cold symptom scores remained fairly constant throughout the year and did not show peaks at  
15 any particular time of year (Figure 2B), although lower scores for cold symptoms were  
16 observed in January-February and July 2012 than at other times of the year.

#### 17 **ED presentations and hospitalizations for asthma in the community**

18 Over the same period in which sampling for the SAVE longitudinal study occurred, asthma-  
19 related emergency department presentations for children aged 5-12 years within the same  
20 geographic region showed two [marked](#) peaks, during February and May 2012 (Figure 2C).  
21 Similar peaks also occurred at the same time in asthma-related hospital admissions of  
22 children 5-12 years old in NSW (Figure 2D). In a time series analysis, we found no detectable  
23 association between the rates of hRV positivity in our cohort and the state-wide hospital  
24 admission rate for asthma in children ( $p=0.7$ ). In these datasets, low numbers of ED

1 presentations and hospital admissions relative to the rest of the observation period were  
2 observed in January-February and July 2012.

3 The relatively mild and consistent temperatures and relative humidity in Sydney over the  
4 observation period are shown for reference in Figure 2E.

### 5 **HRV strain diversity**

6 The diversity of hRV strains found in either nasal or breath samples was examined as a  
7 function of each school term. The highest number of strains was 21 in term 1 (21/53, 40% of  
8 total number of strains detected during the study period), most likely due to the high number  
9 of samples collected (46% (888/1916) of total term-time samples were collected in term 1;  
10 Table S1 in File S1). In term 2 there were 13 strains (13/53, 25% of total number of strains),  
11 in term 3 there were 13 (13/53, 25% of total number of strains) and in term 4 there were eight  
12 (8/53, 15% of total number of strains). Most strains were unique to each school term,  
13 however a small number of strains were detected in both terms 1 and 2 (4/34, 12%), or terms  
14 2 and 3 (3/26, 12%), or terms 3 and 4 (1/21, 5%). One strain (HRV-A01) occurred in term1, 2  
15 and 3.

16 The hRV sequences from viruses infecting children in the SAVE cohort were aligned, and  
17 phylogenetic trees constructed (Figure 3).

18

**Commented [HKR4]:** This is confusing, as you have previously said the peak was in February, and here you are saying it was low in Feb.

## 1 Discussion

2 In this study hRVs occurred throughout the year, with no peak in hRV positive detections  
3 following return to school after the 6-week summer vacation. The children also showed no  
4 peaks in asthma or cold symptoms averaged over two week periods, contrasting with the  
5 finding of two peaks of asthma-related ED presentations to the [hospital from which they were](#)  
6 [recruited SCH](#) and in state-wide hospitalisations, one peak seen after return from the summer  
7 vacation and the other in autumn.

8 In this study, hRV occurred throughout the year (Figure 1) and a similar proportion of nasal  
9 wash samples were positive for hRV during vacation and school terms. Although children  
10 ~~took-collected~~ both a nasal wash and exhaled breath sample, only the prevalence data for  
11 nasal wash samples for comparison with symptoms ~~is-are~~ presented in this paper, because in a  
12 mixed model analysis ~~only~~ the presence of hRV [only](#) in nose but not breath was significantly  
13 associated with worsening asthma symptoms (Tovey et al. 2014). HRVs were the most  
14 commonly identified virus group in this cohort, as has been demonstrated in previous  
15 population studies in young Australian children (Lambert et al., 2007). Australian studies that  
16 only sampled during acute respiratory infections have found peaks of picornavirus (i.e.  
17 predominantly hRV) activity during May (autumn) and October (spring) (Lambert et al.  
18 2007; MacKay et al. 2013), particularly two weeks after each school term commences  
19 (MacKay et al. 2013), however this was not observed in SAVE with routinely-collected  
20 samples. ~~Although there was no back to school peak in hRV detection, h~~ had there been a  
21 back to school peak [in circulating hRV](#), it should have been evident in this study, [given that](#)  
22 [almost half \(46%\) of the total term-time samples were collected in term 1, and 21% of total](#)  
23 [term-time samples were collected in term 2](#). There are a number of epidemiological and  
24 biological phenomena that could account for a lack of back to school effect [in routinely-](#)

Commented [HKR5]: I don't understand this explanation.

1 [collected samples](#), including differences in local climate and behaviour, affecting virus  
2 survival, transmission and circulation patterns (Tovey and Rawlinson 2011).

3  
4 In the SAVE cohort, there were no peaks of day-to-day asthma or cold symptoms, averaged  
5 by fortnight, and there was no detectable association between the distribution of hRV-  
6 positive samples and the state-wide admission rate for asthma in children. This finding was  
7 surprising, as hRV in nasal samples was significantly associated with day-to-day asthma  
8 symptoms in the SAVE cohort (Tovey et al. 2014); however, only five children reported  
9 seeking urgent medical attention for symptoms, and none was admitted to hospital. The  
10 proportions of the two measures of asthma symptoms differed in that relatively more cough  
11 and phlegm were reported in winter than wheeze and chest tightness. This could be due to  
12 cough reported for other reasons including non-viral infections and the effects of winter  
13 climate. By contrast, in community data for severe asthma exacerbations in the same age-  
14 group, as indicated by asthma-related ED presentations in the same geographic area and  
15 hospital admissions in the state as a whole, two peaks were observed. A marked increase in  
16 community ED presentations and hospital admissions for asthma occurred in February and  
17 May 2012, with each of these peaks being approximately four weeks following the return to  
18 school after a vacation. In a study by Lincoln et al 2006, children had an increased risk of  
19 hospitalisation for asthma within the first month after school resumed (Lincoln et al. 2006),  
20 which is in line with the ED and hospital admission data presented in this study.

21 This study detected hRV-A and hRV-C throughout the year. [Previous studies](#) have reported  
22 differences in hRV species seasonality; some studies found prevalence of hRV-A highest in  
23 spring and hRV-C highest in autumn (Miller et al., 2009; Arakawa et al. 2012) while some  
24 studies reported hRV-C to be most common in winter (Linder et al. 2013; MacKay et al.  
25 2013). Olenec et al (Olenec et al. 2010) found hRV-A prevalent in spring, hRV-B prevalent

**Commented [HKR6]:** In this paragraph, should you mention for each study (or all) whether collection was during symptomatic periods?

1 in autumn and hRV-C in both seasons. In this study hRV-B was only detected in the first  
2 half of 2012 (summer and autumn), consistent with Olenec and colleagues' data for autumn  
3 (their study only sampled in spring and autumn, therefore summer data for their cohort ~~is~~[are](#)  
4 unknown) (Olenec et al. 2010). The reason why seasonal patterns of viruses may occur is  
5 complex; it is thought to be a consequence of factors associated with both climate and social  
6 behaviour, interacting with virus biology to affect virus survival and transmission and host  
7 mobility and susceptibility (Sloan et al., 2011; Pica and Bouvier 2012), potentially including  
8 antiviral immunity via Vitamin D which fluctuates seasonally (Camargo et al., 2011).  
9 Meteorological parameters affect virus survival: hRV has greater survival at higher relative  
10 humidity (Sattar et al., 1987), and numbers of children hospitalised with acute respiratory  
11 infections caused by hRV are significantly associated with relative humidity (Du Prel et al.,  
12 2009).

13

14 There are some limitations that must be considered with these data. Because of the logistical  
15 issues related to [twice-weekly](#) sampling and analysis ~~of~~[of](#) data of this nature, the cohort was  
16 relatively small (67 children) with an average sampling time frame of 10 weeks, although  
17 ~~equivalent~~[similar in size](#) to previous studies with routinely-collected samples (Peltola et al.,  
18 2008; Olenec et al. 2010). On average, there were 11 participants collecting samples at any  
19 one period of time. ~~O,~~[O,](#) ~~and~~[and](#) only a single year was studied, which may have limited the power  
20 to identify seasonal variations in prevalence.

21 Sequence data were not available for all samples that were hRV positive, possibly due to low  
22 viral load in some samples. The majority (73.6%) of nasal samples could be sequenced;  
23 other studies have reported success rates of 75-86.9% using the same VP4-VP2 primer set  
24 (Onyango et al., 2012; MacKay et al. 2013). Lower amplification success rates in the VP4-  
25 VP2 region compared to the 5' UTR have been reported in other studies (Lee et al., 2012).

1 This region was chosen for amplification because of its extensive heterogeneity, allowing  
2 type designation (Simmonds et al. 2010). The detection of other viruses (overall 2%) was low  
3 compared to some other studies (e.g. 8.6 % (Olenec et al. 2010) and 13.9% (van der Zalm et  
4 al., 2011)), which could be due to the age of the SAVE cohort, differences in sampling  
5 methods or detection assays.

6  
7 Together, the present findings suggest that different factors contribute to the relationship  
8 between hRV and day-to-day respiratory symptoms compared with the relationship between  
9 hRV and severe asthma exacerbations. One possibility is that children in the SAVE cohort  
10 differed from children presenting to ED or requiring hospitalisation for asthma. Most children  
11 in the SAVE cohort had well-controlled asthma at entry, as indicated by the validated c-ACT  
12 score (Table 1) and ~~self-reported adherence were extremely compliant~~ with ICS medication  
13 throughout the study ~~was high, as shown by records~~. However, no background asthma  
14 control data are available for Australian children with an ED presentation for asthma. We  
15 have reported elsewhere (Tovey et al. 2014) that 42/67 (62.7%) of this cohort were ~~on~~  
16 ~~prescribed~~ regular ICS or ICS/LABA in the previous 12 months. Johnston and colleagues  
17 (Johnston et al. 2005) reported that Canadian children with asthma presenting to the ED were  
18 more likely to have a hRV infection and less likely to have anti-inflammatory medication  
19 prescriptions than children with equally severe asthma in the community who did not present  
20 to the ED. There are several other factors besides acute viral infections that have been  
21 proposed to contribute to back-to-school asthma exacerbations; these include high exposure  
22 to domestic allergens found in school (Almqvist et al., 2001), seasonal outdoor allergens (Im  
23 and Schneider 2005), a preceding 'medication-holiday' (Van Dole et al., 2009), and stress  
24 (Scheuerman et al., 2009).

Commented [HKR7]: By 'records', so you mean the diary data?



1 In summary, this study found that hRVs occur throughout the year in nasal wash samples of  
2 school-age children with asthma. By contrast with asthma-related ED presentations in a  
3 similar geographic area and state-wide hospitalisations, no peak in hRV was observed  
4 following return to school term, and no peaks in asthma or cold symptoms that were related  
5 to back to school or any particular month of the 15 month collection period. This study  
6 suggests that differences in virus prevalence alone may not account for peaks in asthma  
7 symptoms. Identifying the contributing factors may allow the development of interventions to  
8 reduce the current high level of asthma morbidity and health care utilization amongst  
9 children.

10

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5 for c-ACT to be used in this study in accordance with the conditions of GlaxoSmithKline's  
6 licence with QualityMetric.

7

8

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1 **Figure Legends**

2 **Figure 1. Occurrence of viruses in nasal wash samples over the study period.** In each  
3 panel, the y-axis shows the number of positive nasal wash samples/total number of nasal  
4 wash samples expressed as a percentage. Confidence intervals were calculated using Fleiss'  
5 method. 'Other viruses' were influenza A, influenza B, respiratory syncytial virus,  
6 parainfluenza virus 1, 2, 3 and human metapneumovirus. School vacation periods are shown  
7 as dashed lines. Data are analysed by four-weekly intervals from 23 October 2011 to 26  
8 November 2012; no samples were collected between 16 Dec 2011-5 Jan 2012 due to the  
9 vacation season. Sp, spring; Su, summer; Au, autumn and Wi, winter.

10

11 **Figure 2. Chronological distribution of respiratory symptoms in the SAVE cohort, and**  
12 **emergency department presentations/hospitalisations for asthma and meteorological**  
13 **data over the same period.** (A) Fortnightly mean scores of 'wheeze and chest tightness'  
14 (circles) and 'cough and phlegm'(crosses) symptoms (see Methods), and (B) total cold  
15 symptoms for children in the SAVE cohort; and (C) emergency department presentations for  
16 asthma for children aged 5-12 years old in the same geographic region, and (D) asthma  
17 hospitalisations for children aged 5-12 years old in NSW; and (E) temperature (triangles) and  
18 relative humidity (squares). The respiratory symptoms in the SAVE cohort show no  
19 particular peaks throughout the study period, in contrast to the two marked peaks in  
20 emergency department presentations and hospital admissions for asthma.  
21 All data are plotted for the period October 2011-November 2012. School vacation periods are  
22 shown as boxes. Sp, spring; Su, summer; Au, autumn and Wi, winter.

23

24 **Figure 3. Phylogenetic tree of hRV types detected in nasal wash and exhaled breath,**  
25 **demonstrating the large diversity of hRV types infecting the children of the SAVE**  
26 **cohort over the 15 month study period.** Labels show collection date, season and sample  
27 type. Strains are represented the first time they appear for each participant (subsequent  
28 detections of the same strain in the same participant are not shown). N, nasal wash sample; B,  
29 exhaled breath sample; yellow, Summer; pink, Autumn; blue, Winter; green, Spring. Only  
30 bootstrap values over 75 are shown.

31

32

1 **Tables**

2 **Table 1. Characteristics of study participants.**

	Enrolled (N=67)
Age (years), mean $\pm$ SD	8.6 $\pm$ 2.0
Sex, male	64.2% (43/67)
Average number of sample collections	18.7 (range 1-31)
Any atopy on skin prick test	82.2% (53/64)
<b>Emergency Department visits in previous 12 months</b>	
$\geq$ 1 attendance at ED for asthma	9.0% (6/67)
<b>Childhood Asthma Control Test *</b>	
Very poorly controlled ( $\leq$ 12)	1.5% (1/65)
Not well controlled (13-19)	30.8% (20/65)
Well controlled ( $\geq$ 20)	67.7% (44/65)
<b>Virus &amp; Symptoms (average score for all participants)</b>	
HRV positive detections	0.332
Cough & phlegm symptom score (range 0-3)#	0.634
Wheeze & chest tightness symptom score (range 0-3)#	0.390
Total CCQ symptom score (range 0-27)†	2.673

3 \* Childhood Asthma Control Test score ranges from 0 to 27(Liu et al. 2010)

4 # total symptom scores could range from 0 to 6

5 †Common Cold Questionnaire (Powell et al. 2008)

6