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SHORT COMMUNICATION

Many children aged two to five years have a persistent presence of respiratory viruses in their nasopharynx

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On average, young children have six to eight viral respiratory infections (VRI) per year (1). This high prevalence, combined with the ease of spread in the community, makes VRI the most common reason for outpatient visits and hospitalisation in childhood. It is also one of the main reasons that children miss school and their parents miss work to care for them (2). The role of respiratory syncytial viruses (RSV), the parainfluenza virus, influenza virus and adenovirus in causing acute respiratory infections (ARI) in infants and children is well established (1). The importance of some other viruses in the development of respiratory illnesses in children has been clarified relatively recently. In addition to their known role as upper respiratory tract pathogens, rhinoviruses and coronaviruses, often referred to as common cold agents, have been shown to cause lower respiratory tract disease (3,4), whereas the metapneumovirus (5) and human bocavirus (6) have been identified as acute disease causing agents of the respiratory tract.

However, the majority of studies on VRI utilise a cross-sectional design and focus on the relation of a particular virus to the development of a constellation of symptoms. Relatively few studies have dealt with the potential impact of the prolonged presence of a particular virus in the respiratory tract without giving rise to symptoms or with the coexistence of more than one virus (7–10). In the present study, we aimed to prospectively describe the pattern of the presence of respiratory viruses in the upper airways of asymptomatic preschool-aged children living in either urban or rural areas.

The study commenced in December 2010, lasted six months, and included two groups of children aged two to five years. The first group consisted of children attending six day care centres in the rural prefecture of Viotia, which has low levels of air pollution. The second group consisted of children attending four day care centres in the densely populated metropolitan city of Athens, with its heavy air pollution. All of the day care centres were public institutions with about 20 children in each class. All children were eligible to be enrolled in the study, as long as they attended for five days per week and did not suffer from any serious chronic disorder. Nasopharyngeal swabs (NPS) were collected four consecutive times during the school year. Samples were taken at enrolment and then every six to eight weeks. More precisely, the sampling periods were 7–21 December 2010, 24 January to 19 February 2011, 15–28 March 2011 and 11 May to 8 June 2011. The frequent absences of children due to common viral illnesses necessitated long sampling periods and repeated visits to each day care centre during each period, to ensure sampling from all of the participating children. Children were generally well during the sampling, with only some of them having mild nasal congestion, but no other symptoms of common cold.

The sample collection was taken from the posterior nasopharyngeal wall, with the use of a special swab tip made from nylon fibres. This type of thin stylet allowed for a sufficient amount of the sample to be collected without the fibres absorbing the respiratory virus. As a result, it effectively eluted the viral load in the transport medium to enable us to carry out further diagnostic tests. For the transfer and long-term storage, we used Universal Transport Medium (Copan Diagnostics, Murrieta, California, USA), which is considered a reliable system for this purpose.

The samples were tested using CLART PneumoVir (Genomica, Coslada, Madrid, Spain), which is a reverse transcription PCR DNA/RNA assay, for the detection of influenza viruses types A, B and C; parainfluenza viruses types 1, 2, 3 and 4 (subtypes A and B); RSV type A and B; rhinoviruses; metapneumovirus subtypes A and B; enteroviruses (echoviruses); adenoviruses; human coronaviruses subtype 229E; and human bocaviruses.

Total nucleic acid was extracted from 200 μL of each clinical specimen. The detection was based on the amplification of a specific 120- to 330-base pair fragment of the viral genome, using biotinylated primers in a one-tube 5-h reverse transcription PCR amplification protocol. The amplification was followed by hybridisation with specific probes that were immobilised at specific sites of the microarray strip. After incubation with a streptavidin–peroxidase conjugate, the addition of tetramethylbenzidine induced the appearance of an insoluble product that precipitated at the hybridisation sites on the microarray strip. The analysis of the results was processed by a microarray reader, using software provided by the manufacturers, which allowed the fully automatic detection and interpretation of the results, giving a full and specific diagnosis of each analysis in a report. A control was used in each amplification tube to avoid false-negative results.

The differences between groups in prevalence of viral isolation were analysed with Fisher's exact test. The effects that the area where the child lived, had on the recurrent isolation of each distinct virus on the same child, as well as in the number of different viruses present in each sample, was estimated using Poisson regression models with incidence rates ratios (IRRs).

The study was approved by the hospital's ethics committee, and nasopharyngeal samples were collected after informed consent was obtained from the children's parents.

The parents of 89 children did not give their consent, mainly because of fears of distressing or injuring the child during the sampling procedure. There were no children with serious chronic disorders attending the day care centres. The study population finally consisted of 233 children whose parents consented to their participation. Of these, 120 were from the rural area and 113 were from the urban area and the male to female ratios were 64/56 and 60/53 ($p = 0.53$), respectively. The mean and standard deviation ages were 3.5 ± 0.5 and 3.5 ± 0.8 years ($p = 0.88$), for the rural and urban group, respectively.

We took four samples from each child, one during each of the four study periods, and this gave us 932 samples in total. We found that 352 (37.7%) were positive for at least one virus and 680 (52.3%) were negative. Viruses were isolated from 181 to 171 samples from the urban and rural group, respectively ($p = 0.18$). The prevalence of each individual virus is shown in Table 1. The most frequently detected viruses in each one of the four study periods, for the whole study population, were as follows: bocavirus ($n = 71$) for the first, RSV ($n = 38$) for the second, bocavirus ($n = 36$) for the third and rhinovirus ($n = 37$) for the fourth. The most commonly isolated virus in the total of 932 samples was the bocavirus, followed by the rhinovirus and RSV, having been isolated 175, 92 and 74 times, respectively ($p < 0.001$). Many viruses were isolated repeatedly from the same child in sequential sampling periods. The distribution of recurrent and sequential isolations of the same virus in the same child is shown in Table 2. Living in a rural environment was found to exert a protective effect against recurrent presence of bocavirus, with an incidence rate ratio (IRR) of 0.658 and a confidence interval (CI) of 0.457–0.947 ($p = 0.026$). No such effect was detected for the rest of the viruses. In 80 (8.5%) of the total 932 samples, more than one virus was isolated. The exact number and distribution of different viruses isolated concurrently in each child, by sampling period, is shown in Table 3. Isolating more than one virus in each sample was not related to the area where the child lived (IRR 0.912, CI 0.716–1.162, $p = 0.46$).

In general, there were no differences in the total number of viruses isolated in each area. This could be attributed to the same periods of sampling and to the roughly equal

Table 1 Presence of viruses in children from urban (U) and rural (R) areas in four consecutive time periods

	1st period			2nd period			3rd period			4th period		
	U (N = 113)	R (N = 120)	p	U (N = 113)	R (N = 120)	p	U (N = 113)	R (N = 120)	p	U (N = 113)	R (N = 120)	p
Bocavirus	45	26	0.005	21	11	0.040	18	18	ns	19	17	ns
Rhinovirus	13	10	ns	9	10	ns	1	12	0.003	20	17	ns
RSV	2	3	ns	17	21	ns	14	4	0.013	3	10	ns
Influenza virus	2	2	ns	4	18	0.003	1	1	ns	0	0	–
Parainfluenza virus	3	4	ns	2	3	ns	2	2	ns	5	5	ns
Adenovirus	10	2	0.016	1	2	ns	1	0	ns	1	6	ns
Coronavirus	0	0	–	0	0	–	0	0	–	0	0	–
Enterovirus	0	0	–	0	0	–	0	0	–	0	0	–
Metapneumovirus	0	1	ns	0	0	–	1	2	ns	0	0	–
Total	75	48	<0.001	54	65	ns	38	39	ns	48	55	ns

ns = nonsignificant, U = urban area, R = rural area.

Table 2 Viruses repeatedly isolated from nasopharyngeal samples in each child

	Urban areas			Rural areas			
Consecutive sampling periods	2	3	4	2	3	4	p
Bocavirus	24	9	2	12	4	2	0.026
Rhinovirus	8	1	0	11	1	0	0.75
RSV	3	2	0	5	0	0	0.76
Influenza virus	1	1	0	3	1	0	0.18
Parainfluenza virus	3	2	0	5	0	0	0.96
Adenovirus	1	0	0	0	0	0	0.59

The first line of the table denotes the number of consecutive sampling periods that each virus was isolated from the same child. The p values, which represent the differences in the distribution of viruses' recurrent isolation, between urban and rural areas, were calculated with Poisson regression models.

Table 3 Concurrently isolated viruses in each child, by sampling period

	1st period		2nd period		3rd period		4th period		
Number of isolated viruses in each child	2	3	2	3	2	3	2	3	p
Samples from urban areas	12	1	12	2	4	1	8	1	0.46
Samples from rural areas	4	1	14	6	5	0	6	3	

The first line of the table denotes the number of different viruses isolated concurrently in the same child. The p value, which represents the comparison of distributions in the number of isolated viruses in each child per sampling period, between urban and rural areas, was calculated with Poisson regression.

number of children living in rural and urban areas in both groups. The presence of viruses was not related to symptoms, as the children were generally in good health during the sampling period. The most commonly isolated virus in our study was HBoV, and it was the most prevalent in the first and third sampling periods. However, it was second to RSV and rhinovirus in the second and fourth periods, which were epidemic seasons for those two viruses (11,12). The bocavirus was also found to be persistently present in many children, much more so than the rest of the viruses. This is consistent with previous works suggesting prolonged persistence and shedding of bocavirus from the nasopharyngeal secretions in asymptomatic children (13,14). Living in an urban environment was found to facilitate the persistence of bocavirus and, to the best of our knowledge, this is the first time that this relation has been described. It is already known that exposure to air pollution has been linked to childhood respiratory disease and increased hospitalisation from viral causes. So the phenomenon of repeatedly detecting the bocavirus could probably be partly attributed to the higher levels of air pollution and especially to traffic-derived particulate matter, as it has already been described for other viruses (15–17). Apart from bocavirus, the rest of the viruses were isolated in relatively small numbers. The resultant small sample sizes may have prevented our results from showing significant differences in the distributions of other viruses between urban and rural areas. In the analysis, we did not take into account the potential confounding role of indoor air pollution parameters, especially second-hand tobacco smoking, and we consider that this was an important limitation of our study. We did not measure viral load with quantitative PCR or use antibody assays in paired sera so we were not able to study how the bocavirus, or the other viruses, declined so that we could further clarify their role as respiratory pathogens. Likewise, we could not exclude the possibility of reinfection as the cause of the repetitive detection of the bocavirus. Further limitations of our study were the absence of climatic conditions measurements and not having taken into account any preceding clinical symptoms.

CONCLUSION

Our findings suggest that although there were no substantive differences in the carriage of the majority of viruses in the upper airways of preschool-aged children of two to five years of age, with respect to rural or urban residence, the urban environment could have had a promoting effect on the prolonged persistence of HBoV.

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