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Prevalence and seasonality of six respiratory viruses during five consecutive epidemic seasons in Belgium☆,☆☆

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ABSTRACT

Background: Acute Respiratory Infections (ARIs) are a major health problem, especially in young children and the elderly.
Objectives: Insights into the seasonality of respiratory viruses can help us understand when the burden on society is highest and which age groups are most vulnerable.
Study design: We monitored six respiratory viruses during five consecutive seasons (2011–2016) in Belgium. Patient specimens (n = 22876), tested for one or more of the following respiratory viruses, were included in this analysis: Influenza viruses (IAV & IBV), *Human respiratory syncytial virus* (hRSV), *Human metapneumovirus* (hMPV), Adenovirus (ADV) and *Human parainfluenza virus* (hPIV). Data were analysed for four age categories: < 6y, 6–17y, 18–64y and ≥65y.
Results: Children < 6y had the highest infection rates (39% positive vs. 20% positive adults) and the highest frequency of co-infections. hRSV (28%) and IAV (32%) caused the most common respiratory viral infections and followed, like hMPV, a seasonal pattern with winter peaks. hRSV followed an annual pattern with two peaks: first in young children and ± 7 weeks later in elderly. This phenomenon has not been described in literature so far. hPIV and ADV occurred throughout the year with higher rates in winter.
Conclusions: Children < 6y are most vulnerable for respiratory viral infections and have a higher risk for co-infections. hRSV and IAV are the most common respiratory infections with peaks during the winter season in Belgium.

1. Background

Acute respiratory infections (ARIs) are worldwide a major health problem, especially in young children and the elderly [1–3]. They have similar symptoms [4,5], are easily transmitted from one person to the next, and lead to high rates of morbidity, mortality and hospitalisation [6]. According to the WHO, ARIs were in 2015 responsible for 1,8 million deaths in children less than 5 years of age alone [7]. Respiratory viruses, including hRSV, are the leading cause of ARIs [8]. To gain a better understanding of the burden of respiratory viruses on society, it is necessary to learn more about their annual cycles and interactions with each other. Insights into the aetiology of respiratory viral infections can help us understand when the burden on society is highest and which subgroups of the population are most vulnerable for respiratory infections. Combined knowledge should allow a better prediction and management of major outbreaks. Hospitals could for instance anticipate the approaching threat in a pro-active way by vaccinating their staff and the most vulnerable patients in order to limit the spread within the hospital setting.

In this perspective, we analysed the occurrence of several respiratory viruses in Belgium over a 5-year period. Between 2011 and 2016, 22876 specimens from 13298 patients with symptoms of a

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respiratory infection were tested for one or more respiratory viruses as part of the routine clinical practice in the University Hospitals of Leuven. Test results for the following viruses were included in our analysis: Influenza viruses A and B (IAV, IBV), *Human respiratory syncytial virus* (hRSV), *Human metapneumovirus* (hMPV), Adenovirus (ADV) and *Human parainfluenza virus 1–4* (hPIV). The goals of this study are to estimate the prevalence of respiratory viral infections in the studied population, to identify the most common respiratory viruses and their relative frequency over time and to make a comparison between different age groups in the hospitalized population.

2. Study design

2.1. Patient population and data collection

Patients presenting with an ARI in the University Hospitals of Leuven are tested for different respiratory pathogens. For certain well-defined patient groups (e.g. transplant patients), there are clinical care paths in place to determine which tests will be performed at which moments during the treatment. For the majority of the patients presenting with an ARI however there is no pre-defined care path available, and in this case the choice of diagnostic tests depends upon the clinical judgement of the treating physician. In the context of its activities as an associated laboratory to the reference laboratory for respiratory pathogens in Belgium (Antwerp University Hospital), the lab for Clinical and Epidemiological Virology (KU Leuven) collects patient data regarding respiratory viruses [9]. The data used in this study were obtained from the University Hospitals of Leuven. Since the data analyses presented in this study were performed retrospectively and completely anonymous, ethical committee approval was not required.

2.2. Clinical specimens

Mostly oronasopharyngeal swabs, but also bronchoalveolar lavages, sputum and bronchial or endotracheal aspirations were tested for the presence of respiratory viruses.

2.3. Virus detection

The molecular tests performed on the specimens are part of the standard of care in diagnostic procedures of the University Hospitals Leuven. Tests were performed in the Centre for Molecular Diagnostics (CEMOL) of the University Hospital Leuven upon patient- and sample specific request by the treating physician. Therefore, not all patients were tested for the same range of viruses. Influenza A/B and hRSV/hMPV detection were performed by in house developed duplex real-time PCR assays. On rare occasions, urgent samples were tested with the *Binax* quick antigen test for hRSV (Alere) [10]. hPIV was diagnosed with two separate duplex real-time PCRs: hPIV type 1/type 2 and hPIV type 3/type 4. ADV was fluorescently detected with a shell vial assay (cell culture) on Hela cells. In these analyses, test results were defined as positive or negative, no quantitative analyses were performed. Positive results were analysed per week and per age category. Weeks were calculated from Monday to Sunday, according to the week date system of the International Organisation for Standardisation (ISO).

2.4. Data analysis

The age of the tested patients ranged between a few days and 103 years old, which makes it a diverse study population. Patient sample data were divided into 4 age categories: infants and young children (< 6 years old); children and adolescents (6–17 years old); adults (18–64 years old) and elderly (≥65 years old). The investigated period includes 5 consecutive respiratory seasons, starting with the season 2011–2012 and ending with the season 2015–2016. In order to include winter peaks, an epidemic season is defined from week 30 up to week 29 of the following year, with exception of the last season included, 2015–2016, which is defined from week 30 to week 14. The analysis of the 2015–2016 season ended prematurely after week 14 due to a change in diagnostic test at the University Hospitals Leuven. Starting from week 15 of 2016, a new in-house developed respiratory panel assay was introduced in CEMOL. This respiratory panel consists of 12 different multiplex real-time PCRs and detects 28 different respiratory microorganisms, including 5 bacteria, 22 viruses and 1 fungus.

Adenovirus is known to reside in different parts of the human body and to cause different kinds of infection accordingly [11,12]. The non-respiratory samples were therefore excluded from the data set. We calculated the positivity rate per week, the onset, peak and end of each epidemic season, and the median for the 5 consecutive seasons for hRSV, IAV, IBV and hMPV. Additionally, we calculated the number of co-infections with 2 or more respiratory viruses. The onset and end of the epidemic seasons of hRSV, IAV and IBV were defined, respectively, as the first of 2 consecutive weeks with at least 10% positivity rate and the last of two consecutive weeks with less than 10% positivity rate [5]. The onset of the hMPV epidemic season was defined as the first of 2 consecutive weeks with at least 3% positivity rate and the end as the last of 3 consecutive weeks with less than 3% positivity rate [5]. For hPIV and ADV, the onset, end and peak could not be defined.

3. Results

3.1. Incidence and age distribution

Between week 30 of 2011 and week 14 of 2016, 22876 specimens were tested for one or more respiratory viruses to determine the cause of the patients' ARI. An average of 93 tests were performed per week, with peaks during the winter seasons (Fig. 1). Out of the 22876 specimens tested, 6104 (27%) were positive for at least one respiratory virus. The positivity rate of upper respiratory samples (URT) was higher (31%) than the positivity rate of lower respiratory samples (LRT) (14%). The most frequently detected viruses were IAV and hRSV, which were respectively found in 1981 (32%) and 1739 (28%) of the positive samples. Furthermore, 14% of the positive samples contained IBV, 13% hPIV, 9% ADV and 8% hMPV. With exception of the Influenza viruses,

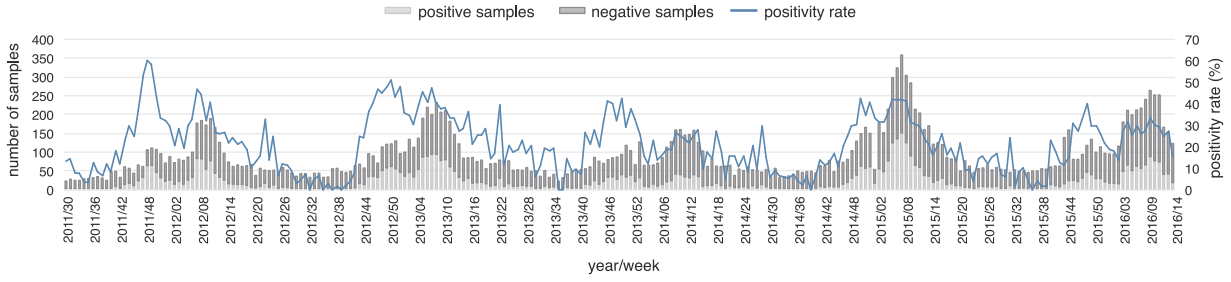

Fig. 1. Weekly number of processed specimens. Positive samples (light grey bars) versus negative samples (dark grey bars) and positivity rate (line graph) per week from week 30 of 2011 until week 14 of 2016. Positive samples can be positive for one or multiple viruses.

Table 1
Positivity rate of 6 respiratory viruses per season during 5 consecutive seasons.

		Number of positive samples	Number of performed tests	Positivity rate (%)
hRSV				
	2011–2012	355	2410	15
	2012–2013	421	3188	13
	2013–2014	313	2576	12
	2014–2015	354	3326	11
	2015–2016	296	2261	13
hMPV				
	2011–2012	99	2405	4
	2012–2013	132	3181	4
	2013–2014	76	2571	3
	2014–2015	96	3325	3
	2015–2016	59	2254	3
ADV				
	2011–2012	114	1858	6
	2012–2013	152	2503	6
	2013–2014	111	2101	5
	2014–2015	115	2521	5
	2015–2016	56	1705	3
	hPIV			
	2011–2012	160	2452	7
	2012–2013	244	3189	8
	2013–2014	174	2320	8
	2014–2015	163	2338	7
	2015–2016	67	870	8
IAV				
	2011–2012	338	2807	12
	2012–2013	294	3857	8
	2013–2014	227	3124	7
	2014–2015	776	4940	16
	2015–2016	346	3847	9
IBV				
	2011–2012	50	2807	2
	2012–2013	378	3857	10
	2013–2014	9	3124	0
	2014–2015	131	4940	3
	2015–2016	295	3847	8

the positivity rate of each virus was consistent over the 5 seasons (Table 1). In the 167 cases for which the same test was performed in an URT and LRT sample, our data do not show a difference in distribution of the detected viruses. The majority of the samples (93–100%) had concordant results for URT and LRT (data not shown). For most viruses the largest group of patients were children under 6 years of age, with the exception of IAV for which most patients were 65 years or older and IBV for which all age groups had a similar incidence (Fig. 2).

3.2. Seasonal distribution

In Fig. 3A, the positive samples per virus are visualised over the 5 seasons. Since the number of positive samples for IAV and hRSV was significantly higher than for the other viruses, the less frequent viruses are visually not well represented in this graph, which is solely based on the number of positive samples per week. Therefore, we calculated the positivity rate for each virus, visualised in Fig. 3B.

Based on the positivity rate, hRSV, hMPV and the Influenza viruses show annual winter cycles between week 44 and week 21, corresponding to the beginning of November up to the end of May of the following year. Looking at the median for the five seasons, the hMPV season had an onset in December at week 50 (SD 3,1), peaked at week 14.5 (SD 4,2) and lasted 27 weeks (SD 4,8) until the median season end in May at week 21 (SD 3,7). hPIV and ADV infections were present throughout the year with a slight downfall during the Belgian summer season (week 25 to week 38). The four subtypes of hPIV had their seasonal peaks at different moments during the year. Subtype 3 had the tendency to have its seasonal peaks around April/May, subtype 4 around December, while subtypes 1 and 2 did not present with a clear seasonal pattern. The number of samples per subtype was too low to define the onset, peak and end of the season (data not shown). There was no clear pattern visible for the IBV infections. In seasons 2012–2013 and 2015–2016, IAV an IBV co-circulated, while the other seasons were dominated by IAV. A mild IBV season was seen in 2011–2012, followed by a strong IBV season in 2012–2013. During the 2013–2014 season, almost no IBV infections were registered. The following year (2014–2015) was again a mild IBV season, followed by a strong 2015–2016 season (Fig. 3.B).

The pronounced annual reoccurring peaks of hRSV and IAV are visualised in Fig. 4. An increase in the hRSV positivity rate was observed in the late fall, indicating the start of the hRSV epidemic season in the late fall with a median seasonal onset at week 44 (SD 1,8). The peak of the hRSV season was on average at week 48 (SD 2,0), which is at the beginning of December (Fig. 4.A). The peak of the hRSV season was followed by the median onset of the IAV season at week 6 (SD 2,9) with a peak in February at week 7 (SD 3,6) (Fig. 4.B). The median end of the hRSV and IAV seasons were at week 5 (SD 2,7) and week 13.5 (SD 1,4) respectively, which makes the hRSV season in Belgium longer than the IAV season (Fig. 4). A detailed overview of the onset, peak, end and duration of the season per year and per virus can be found in Table 3.

Although the onset of hRSV followed a clear annual pattern, a shift in the peak of the hRSV epidemic was noted when the four age groups were separated (Fig. 5). The absolute number of positive samples in young children is much higher than the number of positive samples in the elderly population. To improve the representation of the seasonal peaks, the number of positive samples was normalised for each age category per week by dividing them by the total amount of positive

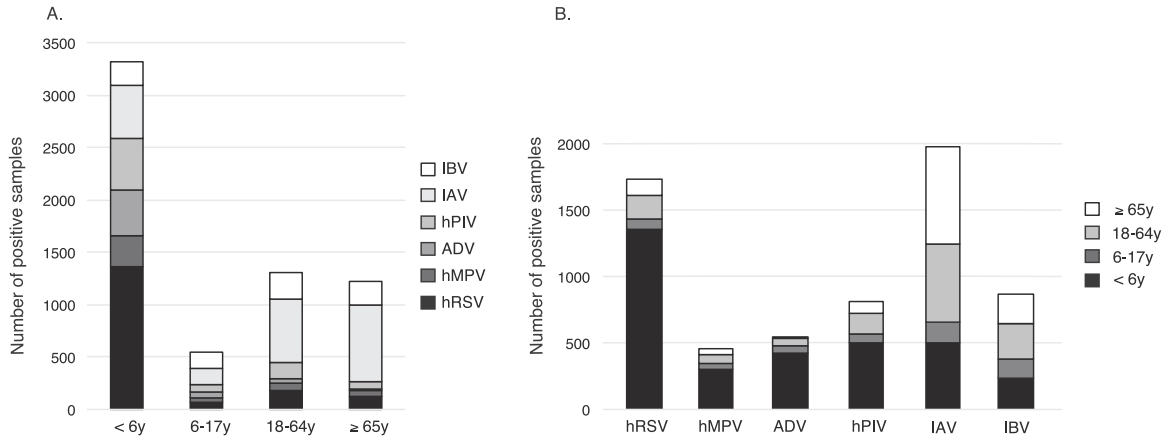

Fig. 2. Absolute number of positive samples per age group (A) and per virus (B).

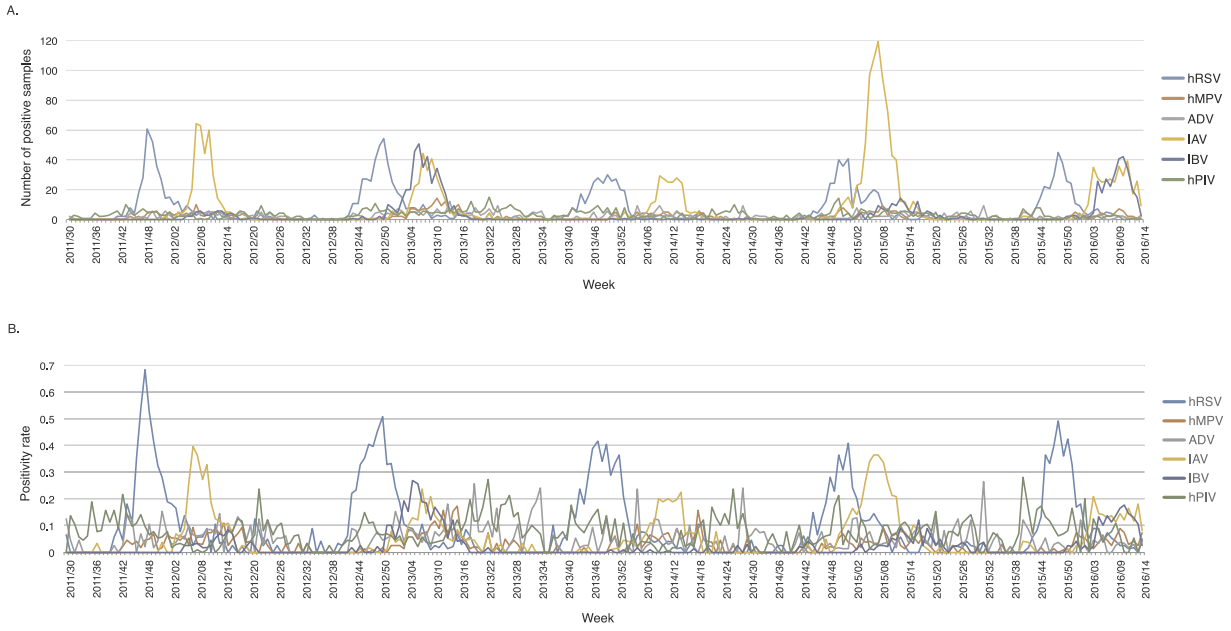

Fig. 3. (A) Occurrence of respiratory viruses per week during 5 consecutive seasons, in absolute numbers. (B) Positivity rate of respiratory viruses per week during 5 consecutive seasons. hRSV (light blue), hMPV (orange), ADV (grey), IAV (yellow), IBV (dark blue) and hPIV (green).

samples for that age category. Based on the relative number of positive samples, the highest peak was seen amongst children < 6 years of age and occurred on average at week 49 (SD 2,0). The peak of the epidemic amongst the elderly (≥65 y) occurred on average 6 weeks later, at week 3 (SD 3,1) (Fig. 5). For the age groups 6–17 years old and 18–64 years old there was no reoccurring pattern in seasonal peaks (not shown). The seasonal peaks of the other respiratory viruses were the same for all age groups.

3.3. Co-infections

Even though not every sample was tested for all viruses, in 284 cases

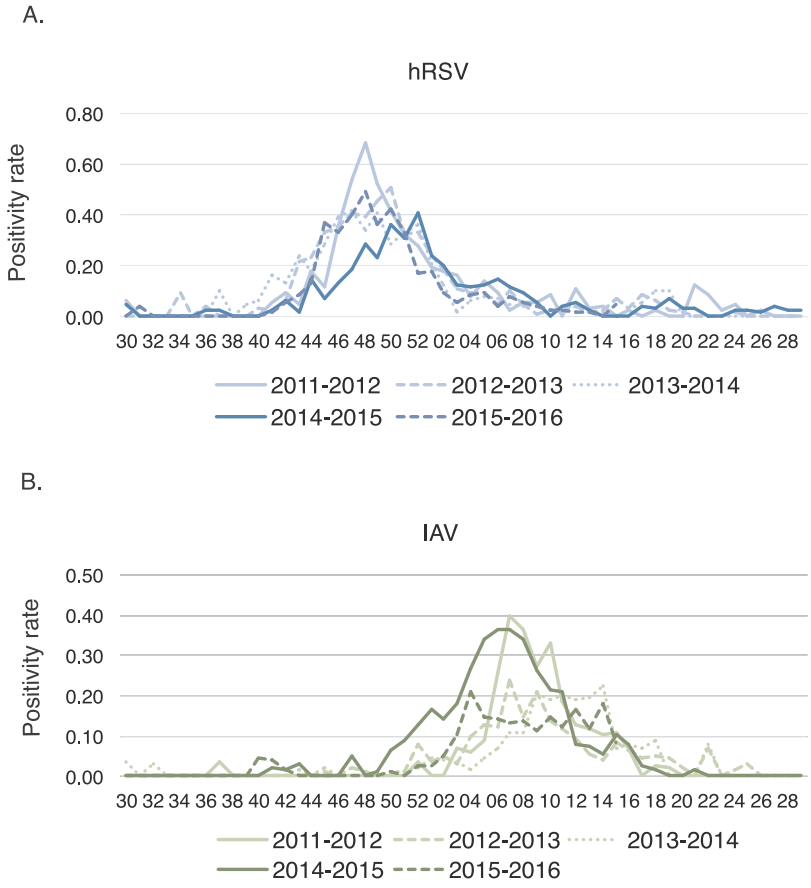

Fig. 4. Annual peaks of *Human respiratory syncytial virus* (A) and Influenza A virus (B), based on the positivity rate.

Table 2
Number of co-infections per respiratory virus in children in comparison to the number of co-infections in the total patient population.

	hMPV		ADV		hPIV		IAV		IBV	
	children	*total*	*children*	*total*	*children*	*total*	*children*	*total*	*children*	*total*
hRSV	15	22	42	45	53	70	8	25	2	6
hMPV			17	20	10	11	8	12	10	12
ADV					23	25	18	18	5	7
hPIV							12	21	1	1
IAV									5	10

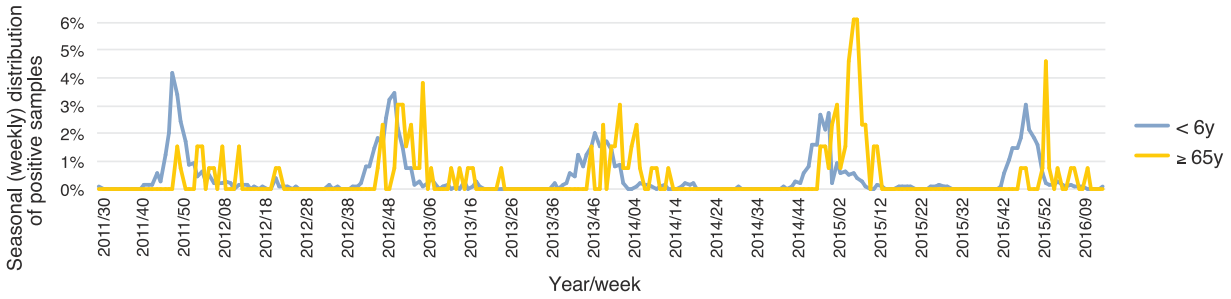

Fig. 5. Epidemic peaks of hRSV in young children (< 6 y) and elderly (≥65 y). The absolute number of positive samples for each age category per week was divided by the total amount of positive samples per age category over the total duration of the study. The seasonal (weekly) distribution of positive samples per age category was plotted over time.

more than one respiratory virus was picked up in a single sample. The majority of these samples (96%) had a dual infection. The combination of hRSV and hPIV was the most common co-infection and was mostly found in young children between 0 and 5 years old (Table 2). 95% of diagnosed co-infections were children under the age of 6. In 9 specimens, 3 respiratory viruses were picked up. These patients all suffered from a hRSV infection, supplemented with 2 other respiratory viral infections: IAV + hPIV (4 patients); hMPV + hPIV; hMPV + ADV; ADV + hPIV; IBV + hPIV and hMPV + IAV (all 1 patient). Only 2 patients, both young children, had a co-infection with 4 different viruses: hRSV + hMPV + ADV + hPIV and hRSV + hMPV + ADV + hPIV.

Table 3
Onset, peak, end and duration of the epidemic season per virus.

		hRSV		hMPV		IAV		IBV	
Onset (week)									
	2011–2012	44	(Nov)	44	(Nov)	6	(Feb)		
	2012–2013	43	(Oct)	52	(Dec)	4	(Jan)	51	(Dec)
	2013–2014	41	(Oct)	50	(Dec)	7	(Feb)		
	2014–2015	46	(Nov)	50	(Dec)	52	(Dec)		
	2015–2016[a]	44	(Nov)	51	(Dec)	2	(Jan)	4	(Jan)
	median 5 seasons	44	(Nov)	50	(Dec)	6	(Feb)	2	(Jan)
	standard deviation	1,8		3,1		2,9		3,5	
Peak (week)									
	2011–2012	48	(Dec)	14	(Apr)	7	(Feb)		
	2012–2013	50	(Dec)	15	(Apr)	7	(Feb)	5	(Feb)
	2013–2014	47	(Nov)	18	(May)	14	(Apr)		
	2014–2015	52	(Dec)	8	(Feb)	6,5	(Feb)		
	2015–2016[a]	48	(Dec)						
	median 5 seasons	48	(Dec)	14,5	(Apr)	7	(Feb)	5	(Feb)
	standard deviation	2,0		4,2		3,6			
End (week)									
	2011–2012	7	(Feb)	21	(May)	14	(Apr)		
	2012–2013	5	(Feb)	20	(May)	13	(Mar)	16	(Apr)
	2013–2014	4	(Jan)	28	(Jul)	16	(Apr)		
	2014–2015	9	(Feb)	21	(May)	13	(Mar)		
	2015–2016[a]	2	(Jan)						
	median 5 seasons	5	(Feb)	21	(May)	13,5	(Mar)	16	(Apr)
	standard deviation	2,7		3,7		1,4			
Duration (number of weeks)									
	2011–2012	16		30		12			
	2012–2013	15		21		10		18	
	2013–2014	16		31		10			
	2014–2015	16		24		14			
	2015–2016[a]	12							
	median 5 seasons	16		27		11		18	
	standard deviation	1,7		4,8		1,9			

[a] Season 2015–2016 ended prematurely at week 15 due to a change in lab test.

4. Discussion

This study examined the occurrence of six respiratory viruses in Belgium between April 2011 and July 2016 in four different age groups: young children (< 6y), children and adolescents (6–17y), adults (18–64y) and elderly (≥65y). All data were obtained from the University Hospitals of Leuven and are therefore a representation of the local epidemiology. The University Hospitals of Leuven is, however, the largest hospital in Belgium and is geographically located in the centre of the country, recruiting patients from a broad surrounding area. Therefore, we assume that our results can be extrapolated to the whole of Belgium. Out of 22876 tested specimens, 6104 (27%) were positive for at least one of the following respiratory viruses: hRSV, IAV, IBV, hMPV, hPIV and/or ADV. In 284 samples (1,2% of all samples and 4,6% of positive samples), more than one respiratory virus was detected. The burden of viral respiratory infections was highest during the winter season. Our findings are in general compatible with other studies for regions with a temperate climate [4,13]. IAV, hRSV and hMPV had an annual epidemic peak during winter. hPIV and ADV were present all year long, but with a higher incidence during winter. Considering the years where an IBV outbreak occurred, IBV had its epidemic peak during winter.

Because of their ability to genetically adapt, Influenza viruses have a regular seasonal circulation and can cause occasional pandemics [14]. In tropical areas, where the climate is warm and humid all year long, IAV circulates throughout the year [8,14–17]. In temperate climates, IAV tends to have annual seasonal peaks during the colder winter months [4,6,18], although bi-annual peaks in late winter/early spring and summer/autumn have been reported [19]. In this study, annual seasonality was observed with epidemic seasons between February and March. IAV was the most frequently detected virus (32% of all positive samples), which is consistent with similar research in Poland [6], China [3,18,20] and Sicily [21]. IAV is followed by hRSV (28%), IBV (14%), hPIV (13%), ADV (9%) and hMPV (8%). In studies in the USA [22] and in Finland [4], however, hRSV was more often detected than IAV. In contrast to the apparent seasonality of IAV, the pattern of IBV is unclear and differs highly in different parts of the world [4,16,19,23]. Our data show a high prevalence during the winter seasons of 2012–2013 and 2015–2016, low prevalence in 2011–2012 and 2014–2015 and almost no prevalence in 2013–2014. A longer follow up period might clarify the IBV circulation pattern in Belgium. The largest groups of Influenza patients are adults and elderly [14,19]. Children however, are more likely to catch an infection with hRSV [1,6,14,20,24]. Exceptions have been reported in Venezuela, where young children were more likely to suffer from an infection with IAV or ADV than hRSV [8].

The seasonality of hRSV is highly variable between different regions in the world [19]. In Belgium, hRSV followed an annual seasonal pattern from November until February, with a median peak in December and a median duration of 16 weeks. These winter peaks are similar to the seasonality of hRSV in other regions with a temperate climate such as Cyprus [24], Finland [4], China [3] and the USA [25]. Gunell and colleagues describe larger hRSV epidemics every other year in Finland [4]. These biennial peaks were not apparent from our data. In tropical climates, the pattern of hRSV seasonality is more variable. In some areas hRSV is present all year round [26,27], in others the incidence of hRSV is limited to the rainy season [15] or has a peak that is unrelated to the weather conditions [8,16,19]. The age-dependent analysis of the hRSV positive samples in Belgium demonstrated that there was a 6-week delay in the epidemic peak in the elderly (≥65y) compared to young children (< 6y), with the peak of the epidemic season for young children at week 49 on average while the peak in the elderly population occurs approximately at week 3. To our knowledge, this phenomenon has not been described yet for hRSV. A possible explanation might be found in the more frequent contact between young children and their grandparents during the Christmas holidays.

Within human pathogens, hMPV is the closest related to hRSV [28], and until recently they were both classified as members of the *Paramyxoviridae* family [28–30]. According to the 2015 virus taxonomy release from the International Committee in Taxonomy of Viruses (ICTV) both viruses are now assigned to the *Pneumoviridae* family [31]. In Belgium, hMPV had an annual pattern of winter peaks and disappearance during summer. The median onset of the hMPV epidemic season was at the end of December and the peak occurred in April, after the hRSV season. In temperate climates, circulation patterns of hMPV are similar to hRSV with winter-to-spring seasonal periods [5,28,32–35]. In the tropical climate of South and Central America, hMPV occurs throughout the whole year [29,36,37]. In Kenya, however, hMPV follows a seasonal pattern from October until April regardless of the tropical climate [38]. In contrast with hRSV, hMPV and the Influenza viruses, not all respiratory viruses present with a seasonal pattern in temperate climates. In Belgium, hPIV occurred all year long due to the different seasonality of the four subtypes with slightly higher peaks during winter. The most common subtype was type 3, which confirms previous research in Central and South America [39] and China [40,41]. ADV was also present all year long, with slightly higher occurrence during the winter months. The seasonality of ADV is similar in temperate and tropical areas [11,12,15,16,19,26].

Previous research has shown that respiratory viruses predominate in young children worldwide, with exception of Influenza [6,20,21,42]. In China, the reported positivity rates of respiratory viruses are twofold higher in children < 5 than in adults [20]. This study confirms that children under the age of 6 are most likely to catch an acute respiratory infection caused by hRSV, hPIV, ADV, IBV and hMPV. The elderly on the other hand are more likely to suffer from an IAV infection. In addition, our data show that young children are the most vulnerable group to suffer from co-infections with multiple respiratory viruses. 95% of all diagnosed co-infections were in patients younger than 6 years old. This might be due to the immature adaptive immune system in children [41]. The most common co-infections involve a hRSV infection combined with another respiratory virus, which is to be expected since hRSV is the most frequent respiratory virus in the group of patients < 6 years old. The combination of hRSV and hPIV is most common. The second most common co-infection is hRSV with ADV.

We provide an overview of the prevalence and seasonality of 6 respiratory viruses causing ARI in different age groups over 5 consecutive respiratory seasons in Belgium. The knowledge obtained in this study can guide physicians in their diagnosis of acute respiratory tract infections. Since symptoms are often very similar, physicians can use the prevalence and seasonality of respiratory viruses as a guide to order laboratory tests. The viruses that are more likely to occur when the patient shows symptoms, can be tested first. More specific laboratory testing will increase time and cost efficiency, which leads to a faster and cheaper diagnosis, improving health management.

Competing interests

Kaat Ramaekers, Els Keyaerts, Annabel Rector, Kurt Beuselinck, Marc Van Ranst and Annie Borremans declare that there is no association that might pose a conflict of interest. Katrien Lagrou reports grants, personal fees and non-financial support from MSD, personal fees and non-financial support from Pfizer, personal fees and non-financial support from Gilead, outside the submitted work.

Ethical approval

Not required.

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