

# Respiratory viruses and influenza in three winter seasons, in Portugal



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## Background:

Respiratory viral disease is a serious public health problem globally with considerable social and economic impacts. Respiratory viruses cause respiratory disease that ranges from mild to severe pneumonia, contributing for morbidity and mortality throughout the world. The etiological diagnosis based on clinical presentation is very difficult due to the similarity of clinical presentation of respiratory viral infections. In order to have a more complete knowledge on the viral etiology of influenza like illness in Portugal, a search for viral agents (including influenza) was done in the framework of the National Influenza Surveillance System, from season 2010/2011 to 2012/2013.

## Material and Methods:

During the 3 winter seasons cases of influenza like illness (ILI) according to the EU case definition were notified by the sentinel and non-sentinel networks. For each studied case a nasopharyngeal swab collected in the first seven days after symptoms onset was received in the National Influenza Reference Laboratory. A multiplex real time PCR for influenza detection was performed for all the samples. All the influenza A were subtyped using the Prodesse Proflu+ (GeneProbe, Inc.), and influenza B lineage was determined using the WHO recommended protocol. The other respiratory viruses: human rhinovirus (hRV), respiratory syncytial virus A and B (RSV A, RSVB), parainfluenza virus 1, 2 and 3 (PIV1, 2, 3), adenovirus (AdV) and metapneumovirus (hMPV) were detected by a multiplex real time PCR, allowing detection of multiple infections. From the first winter, 2010/2011, a random selection of 378 ILI cases (taking into account the time and age distribution of cases representing 37.2% of the reported cases) was analyzed. In the following seasons 2011/2012 and 2012/2013 all samples were tested for influenza and other respiratory viruses, corresponding to 678 cases and 1256 cases, respectively. For the 2012-13 season the odds ratio (OR) of being positive for specific respiratory virus for each sign/symptom was calculated.

## Results:

A total of 2318 samples were analyzed for the 3 seasons. In each season the causal agent was identified in 68%, 52% and 61% of the samples, respectively (Figure 1). The flu virus was the most frequently detected agent accounting for 53.4%, 37.5% and 43.0% of the cases analyzed (Figure 1). In co-circulation with flu, hRV and RSV were detected in the highest percentage of cases after influenza viruses (Figure 1 and 2). hRV was detected throughout the winter in 8-9% of ILI cases, while the RSV circulated simultaneously with the influenza virus and its detection ranged from 2 to 5% of ILI cases (Figure 1 and 2). The PIV, hMPV and AdV virus were detected sporadically. There were cases of mixed infections of 2 or more viruses (representing 1-2%), mostly associated with the influenza virus. In the three seasons the highest percentage of positive cases was detected in children between 0-4 years (Figure 3).

In the 2012-13 season and considering signs and symptoms more associated to Influenza virus infection (Table I), significant association was found with fever (OR=3.13), and cough (OR=3.94). These results were similar if we consider the influenza sub-types A(H1)pdm09 (Fever: OR=2.82; Cough: OR=5.43) and B Yamagata lineage (Fever: OR=2.24; Cough: OR=1.74). Additionally, with A(H1)pdm09 infection was also associated the symptom of Myalgia (OR=2.64). There is a high probability that we are not dealing with a case of flu B/Yamagata (OR=0.44) or even flu (OR=0.70) whenever there is shortness of breath. We also found a strong association between sore throat and the hRV (OR= 2.85) and RSV (OR= 2.23) infections. Shortness of breath was also associated with infection by RSV (OR= 1.77). A negative correlation was found between fever and hRV infection (OR= 0.45).

Chills were the only symptom associated with infection by other respiratory viruses (PIV, AdV and hMPV) (OR= 4.04). At the same time, chills indicate a low probability of infection by hRV (OR= 0.63) (Table I).

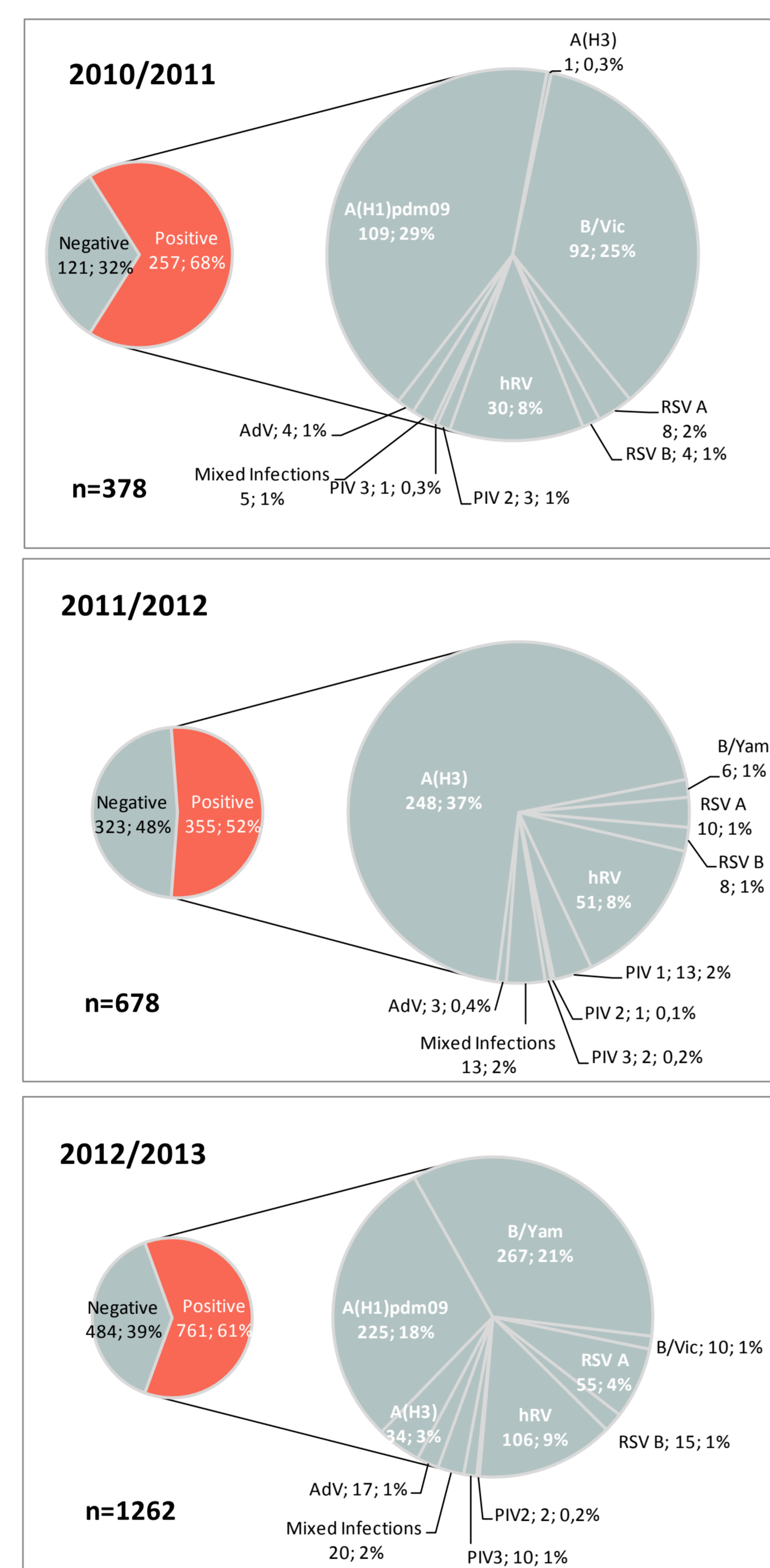


Figure 1 – Number and percentage of flu and respiratory viruses detected in 2010/2011, 2011/2012 and 2012/2013 winter seasons.

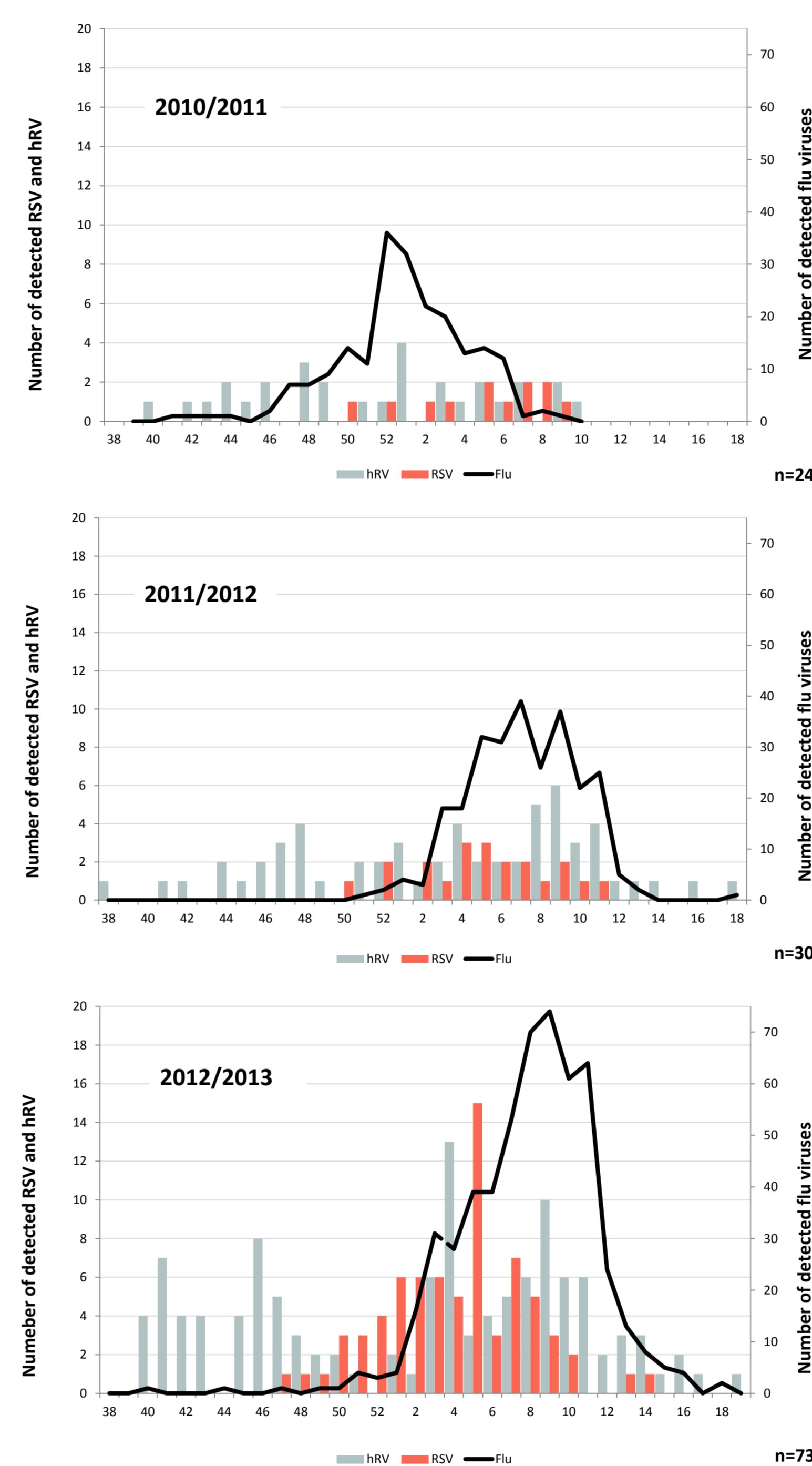


Figure 2 – Detection of hRV, RSV and Flu viruses per week in 2010/2011, 2011/2012 and 2012/2013 winter seasons.

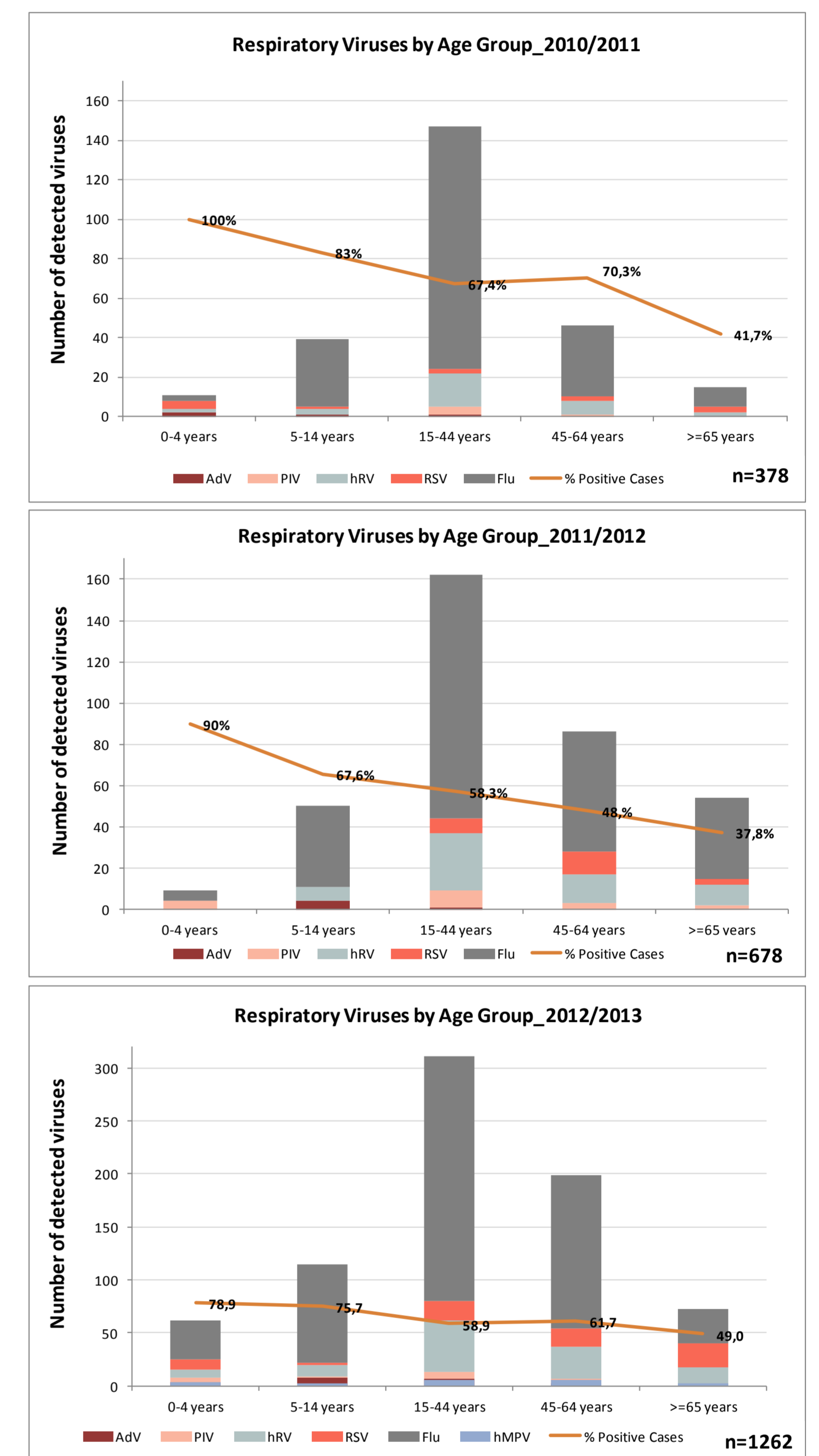


Figure 3 – Percentage of positive cases and number of detected flu and other respiratory virus by age group in 2010/2011, 2011/2012 and 2012/2013 winter seasons.

Table I – Association between the presence of signs and symptoms and a case of infection by flu and other respiratory viruses.

Signs and Symptoms	Flu		Flu A(H1)pdm09		Flu B/Yamagata		RSV		hRV		Other Respiratory Viruses (PIV, AdV and hMPV)	
	Odds ratio	IC95%	Odds ratio	IC95%	Odds ratio	IC95%	Odds ratio	IC95%	Odds ratio	IC95%	Odds ratio	IC95%
Sudden Onset	1,14	(0,82 - 1,58)	1,36	(0,9 - 2,04)	0,74	(0,52 - 1,04)	1,28	(0,65 - 2,55)	1,71	(0,94 - 3,11)	0,92	(0,77 - 1,1)
Fever	<b>3,13</b>	<b>(1,89 - 5,19)</b>	<b>2,82</b>	<b>(1,4 - 5,66)</b>	<b>2,24</b>	<b>(1,23 - 4,07)</b>	0,69	(0,32 - 1,5)	<b>0,45</b>	<b>(0,26 - 0,79)</b>	1,45	(0,5 - 4,22)
Malaise	1,05	(0,68 - 1,64)	1,80	(0,99 - 3,28)	0,70	(0,45 - 1,09)	1,06	(0,45 - 2,51)	0,63	(0,35 - 1,13)	1,43	(0,43 - 4,81)
Headache	1,30	(0,97 - 1,75)	1,25	(0,87 - 1,79)	1,17	(0,84 - 1,64)	1,41	(0,74 - 2,69)	0,83	(0,53 - 1,3)	1,77	(0,76 - 4,1)
Myalgia	1,38	(0,93 - 2,05)	<b>2,64</b>	<b>(1,47 - 4,77)</b>	0,89	(0,6 - 1,34)	0,69	(0,35 - 1,35)	0,73	(0,43 - 1,25)	1,48	(0,51 - 4,29)
Cough	<b>3,94</b>	<b>(2,52 - 6,16)</b>	<b>5,43</b>	<b>(2,36 - 12,48)</b>	<b>1,74</b>	<b>(1,05 - 2,88)</b>	<b>2,83</b>	<b>(0,88 - 9,13)</b>	1,16	(0,6 - 2,21)	1,88	(0,72 - 4,89)
Sore throat	1,14	(0,85 - 1,53)	0,73	(0,52 - 1,02)	0,99	(0,71 - 1,38)	<b>2,23</b>	<b>(1,05 - 4,72)</b>	<b>2,85</b>	<b>(1,51 - 5,39)</b>	1,13	(0,56 - 2,28)
Chills	1,28	(0,94 - 1,74)	1,27	(0,88 - 1,84)	1,05	(0,75 - 1,48)	1,06	(0,58 - 1,95)	<b>0,63</b>	<b>(0,41 - 0,99)</b>	<b>4,04</b>	<b>(1,22 - 13,35)</b>
Shortness of breath	<b>0,70</b>	<b>(0,52 - 0,94)</b>	1,14	(0,81 - 1,59)	<b>0,44</b>	<b>(0,30 - 0,65)</b>	<b>1,77</b>	<b>(1,05 - 2,98)</b>	1,35	(0,88 - 2,09)	1,06	(0,54 - 2,10)

## Conclusions:

The detection of other respiratory viruses enabled the laboratory diagnosis of more than 50 % of ILI cases. The other respiratory viruses accounted for 10-15% of the ILI positives, with greater prevalence for hRV and RSV. Influenza and RSV co-circulated in the 3 winter as described before (Zambon et al., 2001). For the majority of the investigated respiratory virus the presence of one or two signs or symptom was associated to be positive for that virus. These included fever/ cough (Influenza), sore throat (hRV and RSV), shortness of breath (RSV) and chills (PIV, hMPV and AdV virus). The absence of fever and chills was associated to the detection of the hRV.