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## Introduction

Surveillance of SARI has been recommended after the 2009 pandemic to provide critical understanding of the contribution of influenza infection to the burden of disease, and to study the contribution of other viral respiratory pathogens.

The Belgian National Influenza Center monitors seasonal influenza epidemics through a network of sentinel general practitioners. During the 2010-2011 season, surveillance activities were extended to include other respiratory viruses and hospitalized cases of severe acute respiratory infections (SARI) with the objectives (1) to provide timely epidemiological and virological data on SARI, (2) to determine the role of the different respiratory viruses in SARI and (3) to characterize virus strains isolated from most severe SARI cases.

## Study design and methods

### Patients

Nasopharyngeal swabs were collected by hospital emergency wards in SARI patients following the case definition [1]. For each patient, information on gender, age, clinical symptoms, influenza vaccination status, hospitalisation status, antiviral treatment and risk factors was collected. Verbal informed consent was obtained from all participants.

### Methods

- Real time RT-PCR assays were used to detect Influenza A, Influenza B, RSV-A, RSV-B, PIV-1, PIV-2, PIV-3, human metapneumoviruses and rhinoviruses.
- Influenza A strains were subtyped and the lineage of influenza B strains were determined by real time RT-PCR.
- Genomic characterisation of a subset of positive samples was performed by sequencing the hemagglutinin and neuraminidase gene.

## Results

From week 40/2010 to week 20/2011, 540 swabs were collected by the hospital network (Figure 1). The proportion of influenza positive samples increased from week 52/2010 to reach 54% in week 04/2011. RSV was mostly detected from week 46/2010 to week 06/2011 mainly in children below 5 years old. Rhinovirus was present during the whole study period, but levels of hMPV and parainfluenza viruses remained low.

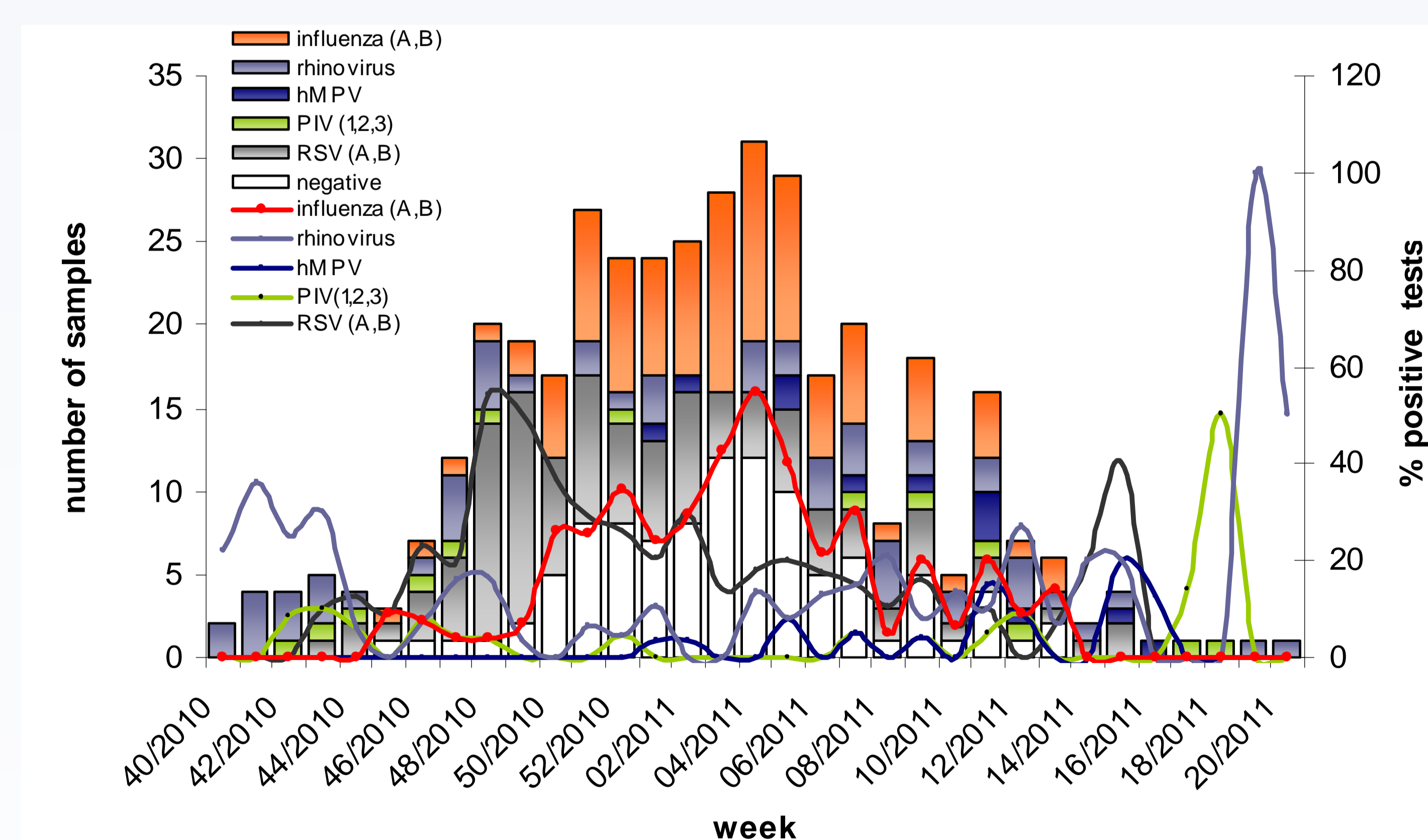


Figure 1. Weekly evolution of number and percentage of respiratory viruses detected in samples from SARI patients from week 40/2010 to week 20/2011.

The distribution of analyzed samples by virus and patient age class is shown in figure 2. Of 522 samples, 259 were positive for at least one virus (50%); including 93 influenza (18%), 87 RSV (17%), 51 rhinovirus (9.8%), 10 hMPV (1.9%) and 14 parainfluenza virus (2.7%). Sixteen co-infections were detected, mostly with rhinoviruses. Most samples were collected from patients below 5 years old (n=228). In this age group, RSV was the most detected virus (36%, with 60% RSV A) while the proportion of influenza was 13% only. Forty-two percent of influenza-positive samples were collected in children below 15 years old with a co-circulation of influenza A and B.

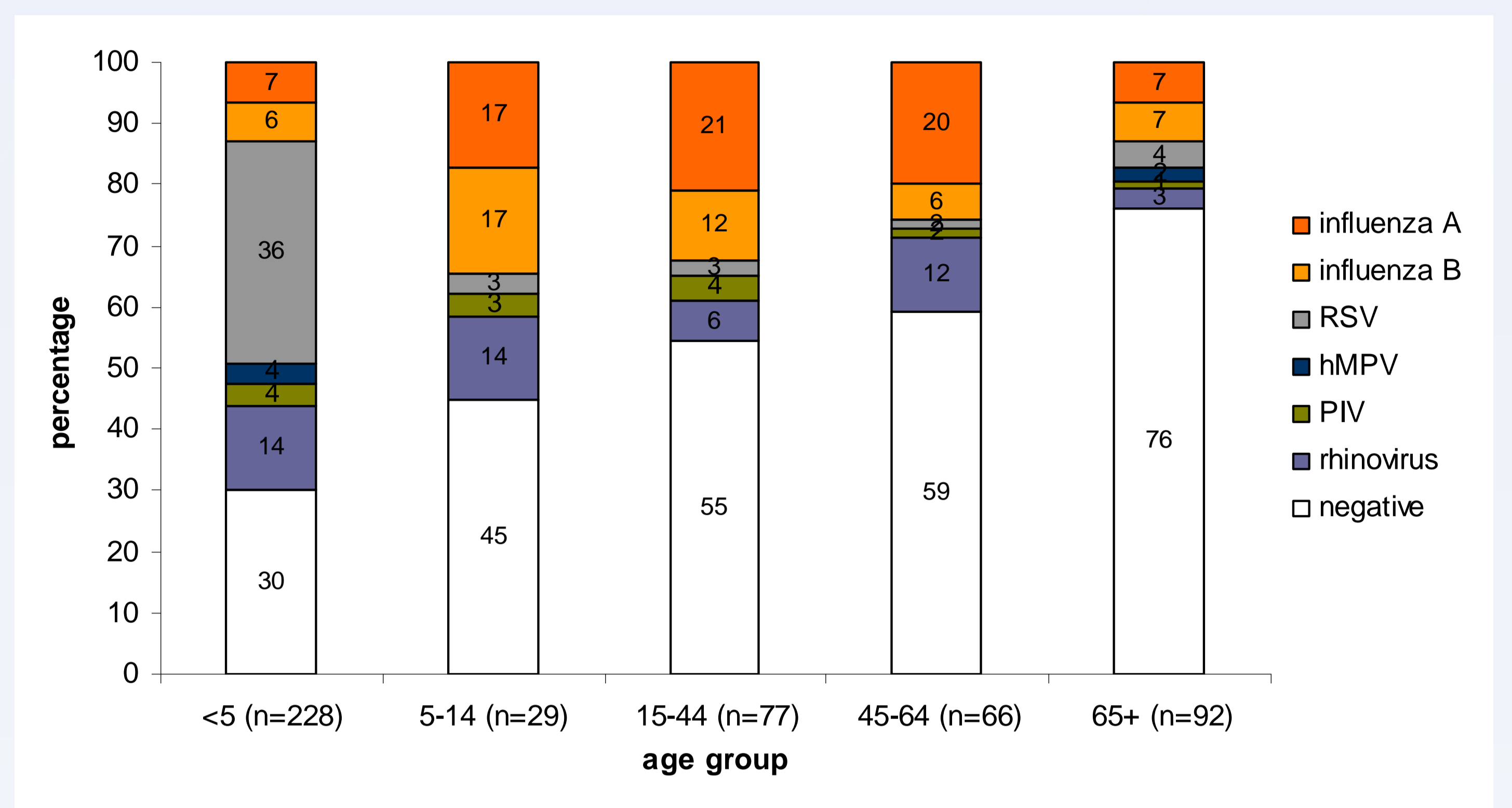


Figure 2. Percentage of samples positive for respiratory viruses by patient age group

Influenza typing revealed 11.5% type A and 7.2% type B among viral isolates. Subtyping analysis of circulating influenza A strains revealed that 47/48 (98%) were the pandemic influenza A(H1N1)2009 variant and one was a A(H3N2) influenza strain. Influenza B strains were mainly from the Victoria lineage (27/31) while 4 were from the Yamagata lineage.

Sequencing of the hemagglutinin gene of influenza A viruses suggested little variation; all strains were closely related to the vaccine strain A/California/7/2009 (Figure 3). Strains from Belgium did not form monophyletic clusters, but were distributed among strains from around the world, suggesting multiple introductions in Belgium. None of the analyzed strains carried known mutations conferring resistance to Oseltamivir nor mutations known to be associated with a higher severity of disease.

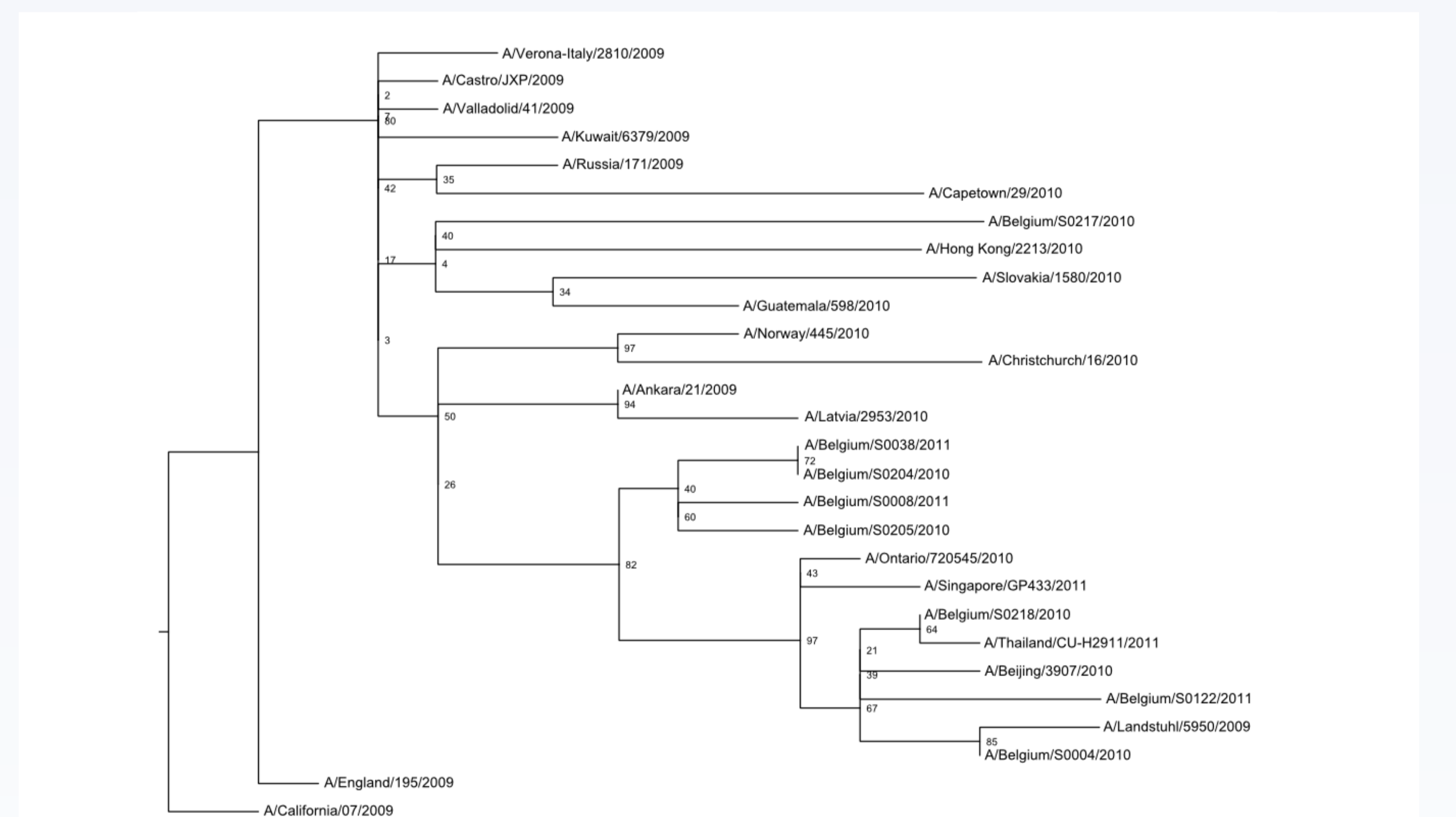


Figure 3. Consensus phylogenetic tree of HA sequences (nt 1-1541) of selected A(H1N1)2009 viruses circulating in Belgium during the 2010-2011 epidemic and reference strains from around the world. Node labels are bootstrap values.

## Conclusions

This pilot study during the 2010-2011 season revealed:

- that the SARI case definition is not specific to influenza, since other viruses were frequently detected as well.
- that viruses of most importance in SARI patients were influenza and RSV, the latter being the most detected virus in children below 5 years old. Also, almost half of all influenza-positive samples were collected from children under 15 years old.
- that in addition to influenza A, also influenza B played a significant role in hospitalisations.
- no known mutations were found associated with antiviral resistance or high pathogenicity.

## References

[1] WHO Regional Office for Europe: Guidance for Influenza Surveillance in Humans.

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