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Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Fischer N, Dauby N, Bossuyt N, et al. Monitoring of human coronaviruses in Belgian primary care and hospitals, 2015–20: a surveillance study. *Lancet Microbe* 2021; published online January 27. https://doi.org/10.1016/S2666-5247(20)30221-4.

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1 SUPPLEMENTARY TABLES:

1.1 SUPPLEMENTARY TABLE 1: TEST RESULTS FROM THE RESPIRATORY VIRUS PANEL OF PRIMARY CARE AND HOSPITAL PATIENT SAMPLES DURING THE WINTER SEASONS IN BELGIUM 2015-2020

	Primary Care, n (%[C])	Hospital, n (%[C])
	Total N = 2,573	**Total N = 6,494**
Coronaviruses		
HCoV-OC43	87 (3·4%)	196 (3·0%)
HCoV-229E	39 (1·5%)	91 (1·4%)
HCoV-NL63	35 (1·4%)	84 (1·3%)
Multiple Coronaviruses	2 (0·1%)	5 (0·1%)
Other Respiratory Viruses		
Influenza viruses	1,328 (51·6%)	2,425 (37·3%)
RSV	87 (3·4%)	752 (11·6%)
Picornaviruses	189 (7·3%)	693 (10·7%)
hMPV	75 (2·9%)	485 (7·5%)
Adenovirus	32 (1·2%)	378 (5·8%)
Bocavirus	5 (0·2%)	178 (2·8%)
Parainfluenza viruses	31 (1·2%)	163 (2·5%)

%[C] Percentage per column

Abbreviations: HCoV = human coronavirus, RSV = respiratory syncytial viruses, hMPV = human metapneumoviruses.

1.2 SUPPLEMENTARY TABLE 2: COINFECTIONS WITH OTHER RESPIRATORY VIRUSES AMONG SEASONAL CORONAVIRUS POSITIVE PATIENTS IN BELGIUM 2015-2020 PER AGE GROUP.

	Primary care sCoV patients, n (%[C])					Hospital sCoV patients, n (%[C])				
	Total (N = 161)	0-4 y/o	5-14 y/o	15-64 y/o (N = 143)	65+ y/o (N = 18)	Total (N = 371)	0-4 y/o (N = 137)	5-14 y/o (N = 10)	15-64 y/o (N = 74)	65+ y/o (N = 150)
Coinfections										
With respiratory viruses	43 (26.7%)	..	..	38 (26.6%)	5 (27.8%)	161 (43.4%)	89 (65.0%)	6 (60.0%)	24 (32.4%)	42 (28.0%)
Other Respiratory Viruses										
Influenza	34 (21.1%)	..	..	30 (21.0%)	4 (22.2%)	67 (18.1%)	19 (13.9%)	4 (40.0%)	17 (23.0%)	27 (18.0%)
RSV	3 (1.9%)	..	..	3 (2.1%)	0 (0.0%)	37 (10.0%)	31 (22.6%)	1 (10.0%)	1 (1.4%)	4 (2.7%)
Picornaviruses	2 (1.2%)	..	..	2 (1.4%)	0 (0.0%)	38 (10.2%)	24 (17.5%)	2 (20.0%)	5 (6.8%)	7 (4.7%)
Adenovirus	0 (0.0%)	..	..	0 (0.0%)	0 (0.0%)	26 (7.0%)	23 (16.8%)	0 (0.0%)	1 (1.4%)	2 (1.3%)
hMPV	4 (2.5%)	..	..	3 (2.1%)		19 (5.1%)	16 (11.7%)	1 (10.0%)	0 (0.0%)	2 (1.3%)
Parainfluenza Viruses	1 (0.6%)	..	..	1 (0.7%)		10 (2.7%)	6 (4.4%)	0 (0.0%)	2 (2.7%)	2 (1.3%)
Bocavirus	1 (0.6%)	..	..	1 (0.7%)		9 (2.5%)	8 (5.8%)	0 (0.0%)	1 (1.4%)	0 (0.0%)

%[C] Percentage per column

Abbreviations: sCoV = seasonal coronavirus, RSV = respiratory syncytial viruses, hMPV = human metapneumoviruses, y/o = years of age.

1.3 Supplementary Table 3: Incidence rate of coronavirus infection per 100.000 person-months per winter season in Belgium 2015-2020.

		Primary care					Hospital				
Coronavirus	**Season**	**Nr of cases**	**Person-months**	**IR**	**Lower 95% CI**	**Upper 95% CI**	**Nr of cases**	**person-months**	**IR**	**Lower 95% CI**	**Upper 95% CI**
HCoV-229E	2015/2016	3	4,136,339	0·1	0·02	0·2	0	3,599,883	0	0	0
	2016/2017	20	3,580,341	0·6	0·4	0·9	29	2,761,569	1·1	0·7	1·5
	2017/2018	0	4,756,185	0	0	0	5	4,050,974	0·1	0·05	0·3
	2018/2019	15	3,644,017	0·4	0·3	0·7	56	3,204,607	1·7	1·4	2·3
	2019/2020	1	2,761,600	0·04	0·01	0·3	1	2,136,405	0	0·01	0·4
HCoV-NL63	2015/2016	2	4,136,339	0·05	0·01	0·2	10	3,599,883	0·3	0·2	0·5
	2016/2017	11	3,580,341	0·3	0·2	0·6	14	2,761,569	0·5	0·3	0·9
	2017/2018	10	4,756,185	0·2	0·1	0·4	35	4,050,974	0·9	0·6	1·2
	2018/2019	5	3,644,017	0·1	0·06	0·3	20	3,204,607	0·6	0·4	1·0
	2019/2020	7	2,761,600	0·3	0·1	0·5	5	2,136,405	0·2	0·1	0·6
HCoV-OC43	2015/2016	29	4,136,339	0·7	0·5	1	33	3,599,883	0·9	0·7	1·3
	2016/2017	20	3,580,341	0·6	0·4	0·9	42	2,761,569	1·5	1·1	2
	2017/2018	13	4,756,185	0·3	0·2	0·5	32	4,050,974	0·8	0·6	1·1
	2018/2019	14	3,644,017	0·4	0·2	0·6	74	3,204,607	2·3	1·8	2·9
	2019/2020	11	2,761,600	0·4	0·2	0·7	15	2,136,405	0·7	0·4	1·2

Abbreviations: HCoV = human coronavirus, Nr = number, IR = incidence rate, CI = confidence interval.

1.4 SUPPLEMENTARY TABLE 4: INCIDENCE RATE OF TOTAL SEASONAL CORONAVIRUS INFECTION, AS WELL AS SPLIT WITHOUT AND WITH COINFECTION, PER AGE GROUP PER 100.000 PERSON-MONTHS IN BELGIUM 2015-2020.

			Primary care					Hospital				
Coronavirus	**Coinfection**	**Age Group**	**Nr of cases**	**Person-months**	**IR**	**Lower 95% CI**	**Upper 95% CI**	**Nr of cases**	**Person-months**	**IR**	**Lower 95% CI**	**Upper 95% CI**
HCoV-229E	Total	0-4 y/o	..	..	..	..	..	12	877,793	1·4	0·8	2·5
		5-14 y/o	..	..	..	..	..	3	1,776,590	0·2	0·1	0·6
		15-64 y/o	36	14,795,205	0·2	0·1	0·3	25	10,149,504	0·2	0·1	0·3
		65+ y/o	3	4,083,277	0·1	0·02	0·3	51	2,949,540	1·7	1·3	2·2
	Yes	0-4 y/o	..	..	..	..	..	10	877,793	1·1	0·6	2·1
		5-14 y/o	..	..	..	..	..	1	1,776,590	0·1	0·01	0·4
		15-64 y/o	15	14,795,205	0·1	0·06	0·17	13	10,149,504	0·1	0·1	0·2
		65+ y/o	2	4,083,277	0·05	0·01	0·2	20	2,949,540	0·7	0·4	1·1
	No	0-4 y/o	..	..	..	..	..	2	877,793	0·2	0·1	0·9
		5-14 y/o	..	..	..	..	..	2	1,776,590	0·1	0·03	0·4
		15-64 y/o	21	14,795,205	0·1	0·09	0·21	12	10,149,504	0·1	0·1	0·2
		65+ y/o	1	4,083,277	0·02	0	0·14	31	2,949,540	1·1	0·7	1·5
HCoV-NL63	Total	0-4 y/o	..	..	..	..	..	46	877,793	5·2	3·9	6·9
		5-14 y/o	..	..	..	..	..	2	1,776,590	0·1	0·03	0·4
		15-64 y/o	31	14,795,205	0·2	0·1	0·3	15	10,149,504	0·1	0·1	0·2
		65+ y/o	4	4,083,277	0·1	0·04	0·3	21	2,949,540	0·7	0·5	1·1
	Yes	0-4 y/o	..	..	..	..	..	25	877,793	2·9	1·9	4·2
		5-14 y/o	..	..	..	..	..	1	1,776,590	0·1	0·01	0·4

		15-64 y/o	6	14,795,205	0·04	0·02	0·09	3	10,149,504	0·03	0·01	0·1
		65+ y/o	1	4,083,277	0·02	0	0·14	5	2,949,540	0·2	0·1	0·4
	No	0-4 y/o	··	··	··	··	··	21	877,793	2·4	1·6	3·7
		5-14 y/o	··	··	··	··	··	1	1,776,590	0·1	0·01	0·4
		15-64 y/o	25	14,795,205	0·17	0·11	0·25	12	10,149,504	0·1	0·1	0·2
		65+ y/o	3	4,083,277	0·07	0·02	0·22	16	2,949,540	0·5	0·3	0·9
HCoV-OC43	Total	0-4 y/o	··	··	··	··	··	79	877,793	9	7·2	11·2
		5-14 y/o	··	··	··	··	··	5	1,776,590	0·3	0·1	0·7
		15-64 y/o	76	14,795,205	0·5	0·4	0·6	34	10,149,504	0·3	0·2	0·4
		65+ y/o	11	4,083,277	0·3	0·2	0·5	78	2,949,540	2·6	2·1	3·2
	Yes	0-4 y/o	··	··	··	··	··	54	877,793	6·2	4·7	8·0
		5-14 y/o	··	··	··	··	··	4	1,776,590	0·2	0·1	0·6
		15-64 y/o	17	14,795,205	0·11	0·07	0·18	8	10,149,504	0·1	0·04	0·2
		65+ y/o	2	4,083,277	0·05	0·01	0·2	17	2,949,540	0·6	0·4	1
	No	0-4 y/o	··	··	··	··	··	25	877,793	2·9	1·9	4·2
		5-14 y/o	··	··	··	··	··	1	1,776,590	0·1	0·01	0·4
		15-64 y/o	59	14,795,205	0·4	0·31	0·52	26	10,149,504	0·3	0·2	0·4
		65+ y/o	9	4,083,277	0·22	0·11	0·42	61	2,949,540	2·1	1·6	2·7

·· no data

Abbreviations: HCoV = human coronavirus, Nr = number, IR = incidence rate, CI = confidence interval, y/o = years of age.

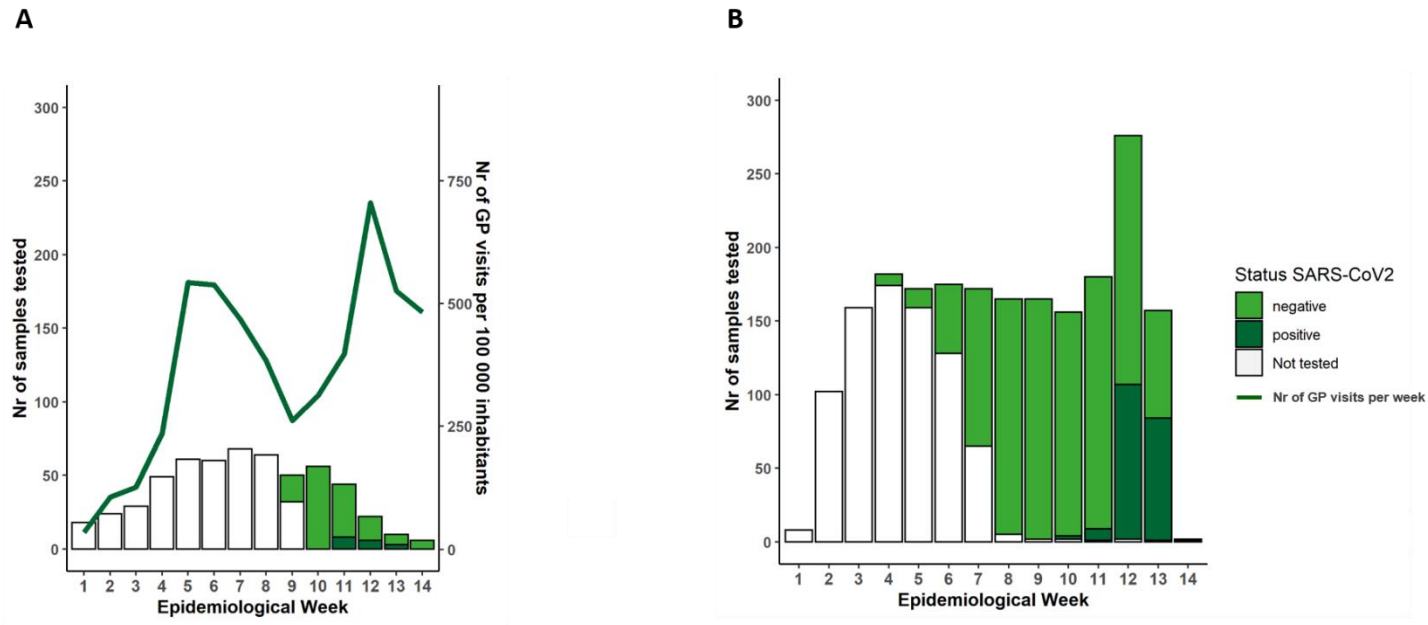
1.5 SUPPLEMENTARY TABLE 5: COMPLICATIONS OR DEATH IN HOSPITALIZED PATIENTS WITH SEASONAL CORONAVIRUS INFECTION WITHOUT AND WITH COINFECTION WITH OTHER RESPIRATORY VIRUSES BY AGE GROUP IN BELGIUM 2015-2020.

	Single HCoV			Coinfection			Chi2* P-value
	Total	**Patients without complications or death, n (%^{R})**	**Patients with complications or death, n (%^{R})**	**Total**	**Patients without complications or death, n (%^{R})**	**Patients with complications or death, n (%^{R})**	
0-4 y/o	39	34 (87·2%)	5 (12·8%)	66	42 (63·6%)	24 (36·4%)	0·017
5-14 y/o	4	1 (25%)	3 (75%)	4	3 (75%)	1 (25%)	
≥ 15 y/o	109	60 (55%)	49 (45%)	40	32 (80%)	8 (20%)	0·0097
Total	152	95 (62·5%)	57 (37·5%)	110	77 (70%)	33 (30%)	

*Pearson's Chi-squared test with Yates' continuity correction
Abbreviations: HCoV = human coronavirus, y/o = years of age.

2 SUPPLEMENTARY FIGURES

2.1 SUPPLEMENTARY FIGURE 1: DETECTION OF SARS-COV2 EMERGENCE THROUGH THE PRIMARY CARE (ILI) (A) AND HOSPITAL (SARI) (B) SENTINEL SURVEILLANCE SYSTEMS IN BELGIUM 2020.



In 2020 the National Influenza Center established testing of samples collected through the primary care (**A**) and hospital (**B**) influenza surveillance systems in Belgium for the presence of SARS-CoV-2 (white bars = not tested, light green bars = negative test, dark green = positive test). The number of visits to the general practitioner network was recorded through the surveillance network (A, green line).

3 SUPPLEMENTARY METHODS

3.1 LABORATORY METHODS AND MATERIALS

3.1.1 PCR primers and probes

Respiratory syncytial virus A	
RSVQA1	GCT CTT AGC AAA GTC AAG TTG AAT GA
RSVQA2	TGC TCC GTT GGA TGG TGT ATT
RSVQA PROBE (HEX)	5HEX/ACA CTC AAC /ZEN/AAA GAT CAA CTT CTG TCA TCC AGC /3IABkFQ
Respiratory syncytial virus B	
RSVQB1	GAT GGC TCT TAG CAA AGT CAA GTT AA
RSVQB2	TGT CAA TAT TAT CTC CTG TAC TAC GTT GAA
RSVQB PROBE (TEX 615)	5TEX615/TGA TAC ATT AAA TAA GGA TCA GCT GCT GTC ATC CA/3BHQ_2
Human metapneumovirus	
hMPV P1	CAT ATA AGC ATG CTA TAT TAA AAG AGT CTC
hMPV P2	CCT ATT TCT GCA GCA TAT TTG TAA TCA G
hMPV PROBE (Cy5)	5Cy5/TGY AAT GAT GAG GGT GTC ACT GCG GTT G/3BHQ_2
Enterovirus	
EV08-1	GGT GCG AAG AGT CTA TTG AGC
EV08-2	CAC CCA AAG TAG TCG GTT CC
EV08p (FAM/ZEN)	56-FAM/CCG GCC CCT /ZEN/GAA TGC /3IABkFQ
Parainfluenza virus 1	
PIV1 X1	TCA CTC TAC CAA CAA CCA CAC A
PIV1 X2	GCC TTC GTG CAA TCT TGT TT
PIV1 PROBE (TEX615)	5TEX615/CAC GAT TCA TGG TGC AGA AGA ACT YGA /3BHQ_2
Parainfluenza virus 2	
PIV2s	CCA TTT ACC TAA GTG ATG GAA
PIV2s	CGT GGC ATA ATC TTC TTT TT
PIV2 PROBE (HEX/ZEN)	5HEX/AAT CGC AAA /ZEN/AGC TGT TCA GTC AC/3IABkFQ
Parainfluenza virus 3	
PIV3 X1	AAA TGA TCT GAT TTA TGC TTA TAC CTC
PIV3 X2	TCA GGT ACC AAG TCT GAG TTT ACA
PIV3 X PROBE (FAM/ZEN)	56-FAM/CGA GGT TGY /ZEN/CAG GAT ATA GGA AAA TCA /3IABkFQ
Parainfluenza virus 4	
F-PIV4	ATG GTG GGA GAY ATT GCA AAA TGG TGG GAG AYA TTG CAA A
R-PIV4	CCA AGC CGA ACT TAA GYG TAA
P-PIV4 (HEX)	5HEX/ATA TAG CYA ATG TCG GAA TGA GYG CGT TCT TT/3BHQ_1
Adenovirus	
AD1	GCC ACG GTG GGG TTT CTA AAC TT
AD2	GCC CCA GTG GTC TTA CAT GCA CAT C
AD PROBE (Cy5)	5Cy5/TGC ACC AGA CCC GGG CTC AGG TAC TCC GA/3BHQ_2
Coronavirus 229E	
Cor229-1	CAG TCA AAT GGG CTG ATG CA
Cor229-2	AAA GGG CTA TAA AGA GAA TAA GGT ATT CT
Cor229- PROBE (TEX615)	5TEX615/CCC TGA CGA CCA CGT TGT GGT TCA /3BHQ_2
Coronavirus OC43	
OC43-1	CGA TGA GGC TAT TCC GAC TAG GT
OC43-2	CCT TCC TGA GCC TTC AAT ATA GTA ACC
OC43- PROBE (HEX/ZEN)	5HEX/TCC GCC TGG /ZEN/CAC GGT ACT CCC T/3IABkFQ
Coronavirus NL63	
NL63 P1	TTG TTC TGT TTT TAA CTT GGT TGC
NL63 P2	CGT TTA GGA GCC TTG GCA AT
NL63 PROBE (Cy5)	5Cy5/TTT ACG TAC TTC TAT TAT GAA GCA TGA /3BHQ_2
Rhinovirus	
F1-Rhv	TGT GAA GAG CCC CGT GTG

F2-Rhv	TGT GAA GAC TCG CAT GTG
R-Rhv	GTA GTC GGT CCC ATC CC
P-RHV (FAM/ZEN)	56-FAM/TCC TCC GGC /ZEN/CCC TGA ATG CG/3IABkFQ
Paraechovirus	
PE1	GTG CCT CTG GGG CCA AAA G
PE2	TCA GAT CCA TAG TGT CGC TTG TTA C
PE- PROBE (TEX 615)	5TEX615/CGA AGG ATG CCC AGA AGG TAC CCG T/3BHQ_2
Bocavirus	
Boca 1A	ACT GGC GTC CAG AAA GAA GA
Boca 2A	TAT GAC GTG GGC TGG ATG TA
Boca A PROBE (Cy5)	5Cy5/TGC ACT CGG GAT GTC ACT GGG /3BHQ_2
Enterovirus D-68	
EV-D68 FOR	TGTTCCCACGGTTGAAAACAA
EV-D68 REV	TGTCTAGCGTCTCATGGTTTTCAC
EV-D69 probe T	/56-FAM/TCC GCT ATA /ZEN/GTA CTT CG/3IABkFQ/
EV-D69 probe A	/56-FAM/ACC GCT ATA /ZEN/GTA CTT CG/3IABkFQ/
Influenza A virus	
InfA Fwd	GACCRATCCTGTCACCTCTGAC
InfA Rev	AGGGCATTYTGGACAAAKCGTCTA
InfA Probe FAM (BHQ-1)	TGCAGTCCTCGCTCACTGGGCACG
Influenza B virus	
InflB Fwd	AAATACGGTGGATTAAATAAAAGCAA
InflB Rev	CCAGCAATAGCTCCGAAGAAA
InflB Probe HEX (BHQ-1)	CACCCATATTGGGCAATTTCCTATGGC
Ribonuclease P	
RP Fwd	AGATTTGGACCTGCGAGCG
RP Rev	GAGCGGCTGTCTCCACAAGT
RP Probe ROX (AbRQSp)	TTCTGACCTGAAGGCTCTGCGCG
Severe acute respiratory syndrome coronavirus 2	
E_Sarbeco_F1	ACAGGTACGTTAATAGTTAATAGCGT
E_Sarbeco_R2	ATATTGCAGCAGTACGCACACA
E_Sarbeco_P1 FAM	ACACTAGCCATCCTTACTGCGCTTCG

3.1.2 PCR reagents and protocol for respiratory virus multiplex RT-qPCR

PCR reagent mix:		
2x PCR Master MIX	12.5 µl	
Primer Mix (5-6 µM primers, 1-2 µM probes)	2.5 µl	
Superscript RT/Platinium Taq	1 µl	
DNA	5 µl	
RNAse free water	adjust to final reaction volume of 25 µl	
PCR program:		
50°C	30 min	45 cycles
94°C	5 min	
94°C	15 sec	
55°C	1 min	

3.1.3 PCR reagents and protocol for SARS-CoV-2 RT-qPCR

PCR reagent mix:		
2x PCR Master MIX	12.5 µl	
E_Sarbeco_F	0.25 µl	
E_Sarbeco_R	0.25 µl	
E_Sarbeco_P	0.5 µl	
RP Fwd	0.25 µl	
RP Rev	0.25 µl	
RP P	0.5 µl	
MgSO4	0.4 µl	
Superscript RT/Platinium Taq	0.5 µl	
RNAse free water	4.6 µl	
DNA	5 µl	
Final reaction volume	**25 µl**	
PCR program:		
55°C	10 min	45 cycles
94°C	3 min	
94°C	15 sec	
58°C	30 sec	

3.1.4 Material and equipment used

Material or equipment	Supplier	Catalog number
PCR primers and probes	Integrated DNA Technologies (IDT), Leuven, Belgium.	
SuperScript™III Platinum® One-Step Quantitative Kit	Invitrogen, Thermo Scientifc Fisher, Waltham, MA, USA	Cat#11732-020 or 11745-100
PCR-grade 8-tubes strips and caps	Invitrogen, Thermo Scientifc Fisher, Waltham, MA, USA	Cat#AM12230
MicroAmp™ Optical 96-Well Reaction Plate	Applied Biosystems, Foster City, CA, USA	Cat# 4306737
1,5 ml reaction tubes	Eppendorf, Hamburg, Germany	Cat#022363204
Table top centrifuge 5425	Eppendorf, Hamburg, Germany	Cat#02231010057
Table top centrifuge 5430 for 96-well plates	Eppendorf, Hamburg, Germany	Cat#022620511
Stratagene Real time PCR Machine (MX 3000P or 3005P)	Agilent Technologies, Santa Clara, CA, USA	Model number: 401513

3.2 SURVEILLANCE DATA COLLECTION AND ANALYSIS

3.2.1 ILI and SARI surveillance in Belgium

Surveillance of ILI was performed every year from week 40 till week 20 through a sentinel system of general practitioners (GP) throughout the country, representing around 1.5% of all Belgian GPs. Patients fulfilling the case definition of acute respiratory infection with onset within the last ten days, with measured fever of ≥38°C and cough and/or dyspnea were eligible to participate in the study. The first two ILI patients belonging to two different households each week were sampled. The ILI surveillance system was active for 32 weeks

each season, except in 2019/2020, where surveillance was interrupted in w13 after 22 weeks due to emergence of SARS-CoV-2 in Belgium.

During the influenza epidemic period (from the last week of December or first/second week of January to the third/last week of April, depending on when influenza virus circulation is detected by the ILI network), SARI surveillance was performed through a network of six sentinel hospitals, two in each administrative region (Flanders, Wallonia and Brussels-Capital). During the study period the weeks of active SARI surveillance in Belgium were as following: 2015/2016: week (w) 01-w17; 2016/2017: w01-w13; 2017/2018: w51-w17; 2018/2019: w01-w15; and 2019/2020: w01-w10. All pediatric and adult patients fulfilling the case definition of acute respiratory infection with onset within the last ten days, with history of fever or measured fever of ≥38°C, with cough and/or dyspnea and overnight hospitalization were eligible for inclusion in the study.

3.2.2 Sample and data collection

Respiratory specimens collected through these networks were sent to the Belgian NIC, along with detailed clinico-epidemiological data, such as information on age, sex, symptoms and potential risk factors such as pregnancy or comorbidities (chronic respiratory diseases, asthma, chronic cardiovascular diseases, renal insufficiency, obesity, diabetes, hepatic or renal insufficiency, immunodeficiency, neuromuscular disease). Hospitalized patients were followed-up for the occurrence of complications (detection of pneumonia based on chest radiography, development of acute respiratory distress syndrome (ARDS), requirement for respiratory assistance and/or for extracorporeal membrane oxygenation (ECMO), admission in intensive care unit (ICU)), or death (all-cause death) during hospitalization.

3.2.3 Determination of the GP and sentinel hospital catchment population

In the GP sentinel network, the catchment population represents the average number of patients that are covered by the GP network per district. To weekly estimate the population covered by the network, the number of inhabitants per active GP (defined as having at least

500 patient contacts/year based on health insurance data) is calculated in each of the 43 districts of Belgium and further multiplied by the number of sentinel practices that participated in each district that week. Only the catchment population of GPs which reported cases to Sciensano were considered per week.

In Belgium, the Federal Public Service provides, the annual market share of hospital admissions of each Belgian general hospital by municipality and age group. The % market share (of registration year – 2) is applied to the each municipalities population and age group. The sum, over all municipalities and sentinel hospitals, provides us with an age-specific catchment population. There are two limitations that may bias the estimate: the market share is estimated on the basis of hospital admissions and not persons. Moreover, only hospitals with a market share of >= 5 % are provided by municipality. The first estimates of this denominator yielded quite plausible catchment populations for each hospital (e.g. in comparison with the number of beds and the regional population structure) and the derived incidences were comparable with estimates based on other (alas equally less than perfect) data sources

3.2.4 Data analysis tools

All data analysis and visualization were performed in R Studio (R version version 3.6.3). The chisq.test and fisher.test function in the stats package, version 4.0.0 were used for statistical analysis. The chisq.test function in R uses a Yates' continuity correction.

Appendix

TABLE OF CONTENTS

1 SUPPLEMENTARY TABLES:

1.1 SUPPLEMENTARY TABLE 1: TEST RESULTS FROM THE RESPIRATORY VIRUS PANEL OF PRIMARY CARE AND HOSPITAL PATIENT SAMPLES DURING THE WINTER SEASONS IN BELGIUM 2015-2020

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HCoV-NL63	35 (1·4%)	84 (1·3%)
Multiple Coronaviruses	2 (0·1%)	5 (0·1%)
Other Respiratory Viruses		
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RSV	87 (3·4%)	752 (11·6%)
Picornaviruses	189 (7·3%)	693 (10·7%)
hMPV	75 (2·9%)	485 (7·5%)
Adenovirus	32 (1·2%)	378 (5·8%)
Bocavirus	5 (0·2%)	178 (2·8%)
Parainfluenza viruses	31 (1·2%)	163 (2·5%)

%[C] Percentage per column

Abbreviations: HCoV = human coronavirus, RSV = respiratory syncytial viruses, hMPV = human metapneumoviruses.

1.2 SUPPLEMENTARY TABLE 2: COINFECTIONS WITH OTHER RESPIRATORY VIRUSES AMONG SEASONAL CORONAVIRUS POSITIVE PATIENTS IN BELGIUM 2015-2020 PER AGE GROUP.

	Primary care sCoV patients, n (%[C])					Hospital sCoV patients, n (%[C])				
	Total (N = 161)	0-4 y/o	5-14 y/o	15-64 y/o (N = 143)	65+ y/o (N = 18)	Total (N = 371)	0-4 y/o (N = 137)	5-14 y/o (N = 10)	15-64 y/o (N = 74)	65+ y/o (N = 150)
Coinfections										
With respiratory viruses	43 (26.7%)	..	..	38 (26.6%)	5 (27.8%)	161 (43.4%)	89 (65.0%)	6 (60.0%)	24 (32.4%)	42 (28.0%)
Other Respiratory Viruses										
Influenza	34 (21.1%)	..	..	30 (21.0%)	4 (22.2%)	67 (18.1%)	19 (13.9%)	4 (40.0%)	17 (23.0%)	27 (18.0%)
RSV	3 (1.9%)	..	..	3 (2.1%)	0 (0.0%)	37 (10.0%)	31 (22.6%)	1 (10.0%)	1 (1.4%)	4 (2.7%)
Picornaviruses	2 (1.2%)	..	..	2 (1.4%)	0 (0.0%)	38 (10.2%)	24 (17.5%)	2 (20.0%)	5 (6.8%)	7 (4.7%)
Adenovirus	0 (0.0%)	..	..	0 (0.0%)	0 (0.0%)	26 (7.0%)	23 (16.8%)	0 (0.0%)	1 (1.4%)	2 (1.3%)
hMPV	4 (2.5%)	..	..	3 (2.1%)		19 (5.1%)	16 (11.7%)	1 (10.0%)	0 (0.0%)	2 (1.3%)
Parainfluenza Viruses	1 (0.6%)	..	..	1 (0.7%)		10 (2.7%)	6 (4.4%)	0 (0.0%)	2 (2.7%)	2 (1.3%)
Bocavirus	1 (0.6%)	..	..	1 (0.7%)		9 (2.5%)	8 (5.8%)	0 (0.0%)	1 (1.4%)	0 (0.0%)

%[C] Percentage per column

Abbreviations: sCoV = seasonal coronavirus, RSV = respiratory syncytial viruses, hMPV = human metapneumoviruses, y/o = years of age.

1.3 Supplementary Table 3: Incidence rate of coronavirus infection per 100.000 person-months per winter season in Belgium 2015-2020.

		Primary care					Hospital				
Coronavirus	**Season**	**Nr of cases**	**Person-months**	**IR**	**Lower 95% CI**	**Upper 95% CI**	**Nr of cases**	**person-months**	**IR**	**Lower 95% CI**	**Upper 95% CI**
HCoV-229E	2015/2016	3	4,136,339	0·1	0·02	0·2	0	3,599,883	0	0	0
	2016/2017	20	3,580,341	0·6	0·4	0·9	29	2,761,569	1·1	0·7	1·5
	2017/2018	0	4,756,185	0	0	0	5	4,050,974	0·1	0·05	0·3
	2018/2019	15	3,644,017	0·4	0·3	0·7	56	3,204,607	1·7	1·4	2·3
	2019/2020	1	2,761,600	0·04	0·01	0·3	1	2,136,405	0	0·01	0·4
HCoV-NL63	2015/2016	2	4,136,339	0·05	0·01	0·2	10	3,599,883	0·3	0·2	0·5
	2016/2017	11	3,580,341	0·3	0·2	0·6	14	2,761,569	0·5	0·3	0·9
	2017/2018	10	4,756,185	0·2	0·1	0·4	35	4,050,974	0·9	0·6	1·2
	2018/2019	5	3,644,017	0·1	0·06	0·3	20	3,204,607	0·6	0·4	1·0
	2019/2020	7	2,761,600	0·3	0·1	0·5	5	2,136,405	0·2	0·1	0·6
HCoV-OC43	2015/2016	29	4,136,339	0·7	0·5	1	33	3,599,883	0·9	0·7	1·3
	2016/2017	20	3,580,341	0·6	0·4	0·9	42	2,761,569	1·5	1·1	2
	2017/2018	13	4,756,185	0·3	0·2	0·5	32	4,050,974	0·8	0·6	1·1
	2018/2019	14	3,644,017	0·4	0·2	0·6	74	3,204,607	2·3	1·8	2·9
	2019/2020	11	2,761,600	0·4	0·2	0·7	15	2,136,405	0·7	0·4	1·2

Abbreviations: HCoV = human coronavirus, Nr = number, IR = incidence rate, CI = confidence interval.

1.4 SUPPLEMENTARY TABLE 4: INCIDENCE RATE OF TOTAL SEASONAL CORONAVIRUS INFECTION, AS WELL AS SPLIT WITHOUT AND WITH COINFECTION, PER AGE GROUP PER 100.000 PERSON-MONTHS IN BELGIUM 2015-2020.

			Primary care					Hospital				
Coronavirus	**Coinfection**	**Age Group**	**Nr of cases**	**Person-months**	**IR**	**Lower 95% CI**	**Upper 95% CI**	**Nr of cases**	**Person-months**	**IR**	**Lower 95% CI**	**Upper 95% CI**
HCoV-229E	Total	0-4 y/o	..	..	..	..	..	12	877,793	1·4	0·8	2·5
		5-14 y/o	..	..	..	..	..	3	1,776,590	0·2	0·1	0·6
		15-64 y/o	36	14,795,205	0·2	0·1	0·3	25	10,149,504	0·2	0·1	0·3
		65+ y/o	3	4,083,277	0·1	0·02	0·3	51	2,949,540	1·7	1·3	2·2
	Yes	0-4 y/o	..	..	..	..	..	10	877,793	1·1	0·6	2·1
		5-14 y/o	..	..	..	..	..	1	1,776,590	0·1	0·01	0·4
		15-64 y/o	15	14,795,205	0·1	0·06	0·17	13	10,149,504	0·1	0·1	0·2
		65+ y/o	2	4,083,277	0·05	0·01	0·2	20	2,949,540	0·7	0·4	1·1
	No	0-4 y/o	..	..	..	..	..	2	877,793	0·2	0·1	0·9
		5-14 y/o	..	..	..	..	..	2	1,776,590	0·1	0·03	0·4
		15-64 y/o	21	14,795,205	0·1	0·09	0·21	12	10,149,504	0·1	0·1	0·2
		65+ y/o	1	4,083,277	0·02	0	0·14	31	2,949,540	1·1	0·7	1·5
HCoV-NL63	Total	0-4 y/o	..	..	..	..	..	46	877,793	5·2	3·9	6·9
		5-14 y/o	..	..	..	..	..	2	1,776,590	0·1	0·03	0·4
		15-64 y/o	31	14,795,205	0·2	0·1	0·3	15	10,149,504	0·1	0·1	0·2
		65+ y/o	4	4,083,277	0·1	0·04	0·3	21	2,949,540	0·7	0·5	1·1
	Yes	0-4 y/o	..	..	..	..	..	25	877,793	2·9	1·9	4·2
		5-14 y/o	..	..	..	..	..	1	1,776,590	0·1	0·01	0·4

		15-64 y/o	6	14,795,205	0·04	0·02	0·09	3	10,149,504	0·03	0·01	0·1
		65+ y/o	1	4,083,277	0·02	0	0·14	5	2,949,540	0·2	0·1	0·4
	No	0-4 y/o	··	··	··	··	··	21	877,793	2·4	1·6	3·7
		5-14 y/o	··	··	··	··	··	1	1,776,590	0·1	0·01	0·4
		15-64 y/o	25	14,795,205	0·17	0·11	0·25	12	10,149,504	0·1	0·1	0·2
		65+ y/o	3	4,083,277	0·07	0·02	0·22	16	2,949,540	0·5	0·3	0·9
HCoV-OC43	Total	0-4 y/o	··	··	··	··	··	79	877,793	9	7·2	11·2
		5-14 y/o	··	··	··	··	··	5	1,776,590	0·3	0·1	0·7
		15-64 y/o	76	14,795,205	0·5	0·4	0·6	34	10,149,504	0·3	0·2	0·4
		65+ y/o	11	4,083,277	0·3	0·2	0·5	78	2,949,540	2·6	2·1	3·2
	Yes	0-4 y/o	··	··	··	··	··	54	877,793	6·2	4·7	8·0
		5-14 y/o	··	··	··	··	··	4	1,776,590	0·2	0·1	0·6
		15-64 y/o	17	14,795,205	0·11	0·07	0·18	8	10,149,504	0·1	0·04	0·2
		65+ y/o	2	4,083,277	0·05	0·01	0·2	17	2,949,540	0·6	0·4	1
	No	0-4 y/o	··	··	··	··	··	25	877,793	2·9	1·9	4·2
		5-14 y/o	··	··	··	··	··	1	1,776,590	0·1	0·01	0·4
		15-64 y/o	59	14,795,205	0·4	0·31	0·52	26	10,149,504	0·3	0·2	0·4
		65+ y/o	9	4,083,277	0·22	0·11	0·42	61	2,949,540	2·1	1·6	2·7

·· no data

Abbreviations: HCoV = human coronavirus, Nr = number, IR = incidence rate, CI = confidence interval, y/o = years of age.

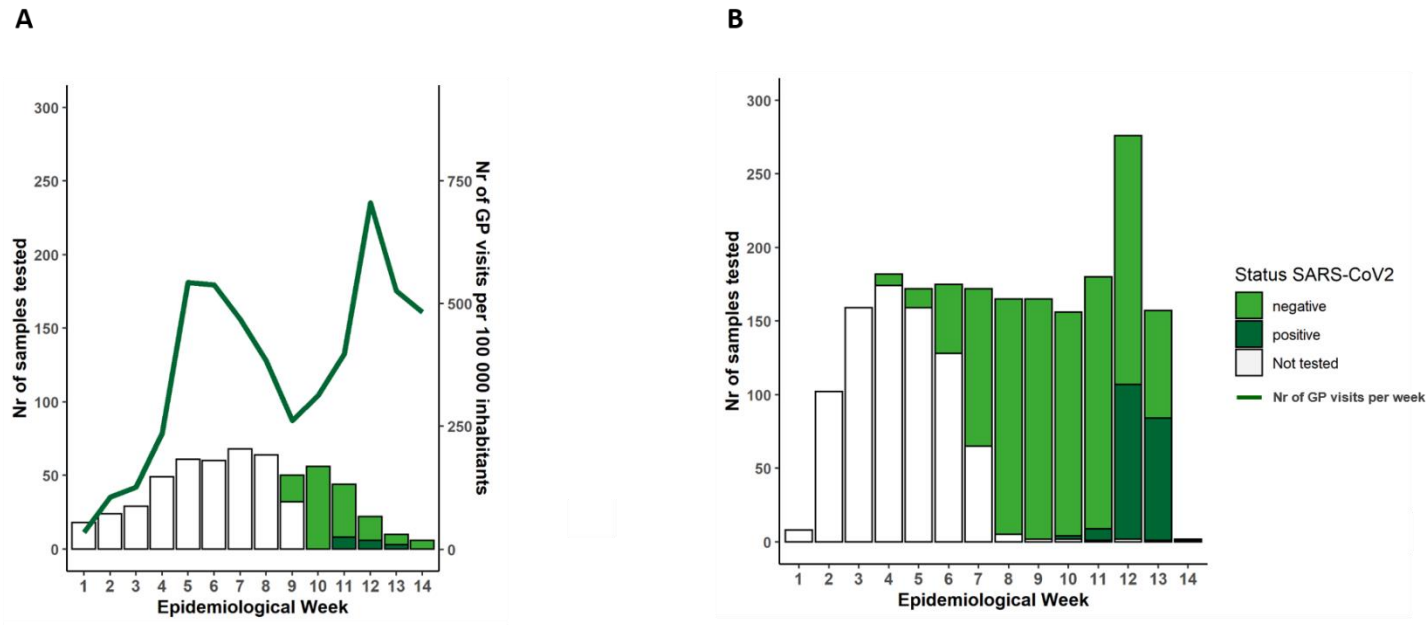
1.5 SUPPLEMENTARY TABLE 5: COMPLICATIONS OR DEATH IN HOSPITALIZED PATIENTS WITH SEASONAL CORONAVIRUS INFECTION WITHOUT AND WITH COINFECTION WITH OTHER RESPIRATORY VIRUSES BY AGE GROUP IN BELGIUM 2015-2020.

	Single HCoV			Coinfection			Chi2* P-value
	Total	**Patients without complications or death, n (%^{R})**	**Patients with complications or death, n (%^{R})**	**Total**	**Patients without complications or death, n (%^{R})**	**Patients with complications or death, n (%^{R})**	
0-4 y/o	39	34 (87·2%)	5 (12·8%)	66	42 (63·6%)	24 (36·4%)	0·017
5-14 y/o	4	1 (25%)	3 (75%)	4	3 (75%)	1 (25%)	
≥ 15 y/o	109	60 (55%)	49 (45%)	40	32 (80%)	8 (20%)	0·0097
Total	152	95 (62·5%)	57 (37·5%)	110	77 (70%)	33 (30%)	

*Pearson's Chi-squared test with Yates' continuity correction
Abbreviations: HCoV = human coronavirus, y/o = years of age.

2 SUPPLEMENTARY FIGURES

2.1 SUPPLEMENTARY FIGURE 1: DETECTION OF SARS-COV2 EMERGENCE THROUGH THE PRIMARY CARE (ILI) (A) AND HOSPITAL (SARI) (B) SENTINEL SURVEILLANCE SYSTEMS IN BELGIUM 2020.



In 2020 the National Influenza Center established testing of samples collected through the primary care (**A**) and hospital (**B**) influenza surveillance systems in Belgium for the presence of SARS-CoV-2 (white bars = not tested, light green bars = negative test, dark green = positive test). The number of visits to the general practitioner network was recorded through the surveillance network (A, green line).

3 SUPPLEMENTARY METHODS

3.1 LABORATORY METHODS AND MATERIALS

3.1.1 PCR primers and probes

Respiratory syncytial virus A	
RSVQA1	GCT CTT AGC AAA GTC AAG TTG AAT GA
RSVQA2	TGC TCC GTT GGA TGG TGT ATT
RSVQA PROBE (HEX)	5HEX/ACA CTC AAC /ZEN/AAA GAT CAA CTT CTG TCA TCC AGC /3IABkFQ
Respiratory syncytial virus B	
RSVQB1	GAT GGC TCT TAG CAA AGT CAA GTT AA
RSVQB2	TGT CAA TAT TAT CTC CTG TAC TAC GTT GAA
RSVQB PROBE (TEX 615)	5TEX615/TGA TAC ATT AAA TAA GGA TCA GCT GCT GTC ATC CA/3BHQ_2
Human metapneumovirus	
hMPV P1	CAT ATA AGC ATG CTA TAT TAA AAG AGT CTC
hMPV P2	CCT ATT TCT GCA GCA TAT TTG TAA TCA G
hMPV PROBE (Cy5)	5Cy5/TGY AAT GAT GAG GGT GTC ACT GCG GTT G/3BHQ_2
Enterovirus	
EV08-1	GGT GCG AAG AGT CTA TTG AGC
EV08-2	CAC CCA AAG TAG TCG GTT CC
EV08p (FAM/ZEN)	56-FAM/CCG GCC CCT /ZEN/GAA TGC /3IABkFQ
Parainfluenza virus 1	
PIV1 X1	TCA CTC TAC CAA CAA CCA CAC A
PIV1 X2	GCC TTC GTG CAA TCT TGT TT
PIV1 PROBE (TEX615)	5TEX615/CAC GAT TCA TGG TGC AGA AGA ACT YGA /3BHQ_2
Parainfluenza virus 2	
PIV2s	CCA TTT ACC TAA GTG ATG GAA
PIV2s	CGT GGC ATA ATC TTC TTT TT
PIV2 PROBE (HEX/ZEN)	5HEX/AAT CGC AAA /ZEN/AGC TGT TCA GTC AC/3IABkFQ
Parainfluenza virus 3	
PIV3 X1	AAA TGA TCT GAT TTA TGC TTA TAC CTC
PIV3 X2	TCA GGT ACC AAG TCT GAG TTT ACA
PIV3 X PROBE (FAM/ZEN)	56-FAM/CGA GGT TGY /ZEN/CAG GAT ATA GGA AAA TCA /3IABkFQ
Parainfluenza virus 4	
F-PIV4	ATG GTG GGA GAY ATT GCA AAA TGG TGG GAG AYA TTG CAA A
R-PIV4	CCA AGC CGA ACT TAA GYG TAA
P-PIV4 (HEX)	5HEX/ATA TAG CYA ATG TCG GAA TGA GYG CGT TCT TT/3BHQ_1
Adenovirus	
AD1	GCC ACG GTG GGG TTT CTA AAC TT
AD2	GCC CCA GTG GTC TTA CAT GCA CAT C
AD PROBE (Cy5)	5Cy5/TGC ACC AGA CCC GGG CTC AGG TAC TCC GA/3BHQ_2
Coronavirus 229E	
Cor229-1	CAG TCA AAT GGG CTG ATG CA
Cor229-2	AAA GGG CTA TAA AGA GAA TAA GGT ATT CT
Cor229- PROBE (TEX615)	5TEX615/CCC TGA CGA CCA CGT TGT GGT TCA /3BHQ_2
Coronavirus OC43	
OC43-1	CGA TGA GGC TAT TCC GAC TAG GT
OC43-2	CCT TCC TGA GCC TTC AAT ATA GTA ACC
OC43- PROBE (HEX/ZEN)	5HEX/TCC GCC TGG /ZEN/CAC GGT ACT CCC T/3IABkFQ
Coronavirus NL63	
NL63 P1	TTG TTC TGT TTT TAA CTT GGT TGC
NL63 P2	CGT TTA GGA GCC TTG GCA AT
NL63 PROBE (Cy5)	5Cy5/TTT ACG TAC TTC TAT TAT GAA GCA TGA /3BHQ_2
Rhinovirus	
F1-Rhv	TGT GAA GAG CCC CGT GTG

F2-Rhv	TGT GAA GAC TCG CAT GTG
R-Rhv	GTA GTC GGT CCC ATC CC
P-RHV (FAM/ZEN)	56-FAM/TCC TCC GGC /ZEN/CCC TGA ATG CG/3IABkFQ
Paraechovirus	
PE1	GTG CCT CTG GGG CCA AAA G
PE2	TCA GAT CCA TAG TGT CGC TTG TTA C
PE- PROBE (TEX 615)	5TEX615/CGA AGG ATG CCC AGA AGG TAC CCG T/3BHQ_2
Bocavirus	
Boca 1A	ACT GGC GTC CAG AAA GAA GA
Boca 2A	TAT GAC GTG GGC TGG ATG TA
Boca A PROBE (Cy5)	5Cy5/TGC ACT CGG GAT GTC ACT GGG /3BHQ_2
Enterovirus D-68	
EV-D68 FOR	TGTTCCCACGGTTGAAAACAA
EV-D68 REV	TGTCTAGCGTCTCATGGTTTTCAC
EV-D69 probe T	/56-FAM/TCC GCT ATA /ZEN/GTA CTT CG/3IABkFQ/
EV-D69 probe A	/56-FAM/ACC GCT ATA /ZEN/GTA CTT CG/3IABkFQ/
Influenza A virus	
InfA Fwd	GACCRATCCTGTCACCTCTGAC
InfA Rev	AGGGCATTYTGGACAAAKCGTCTA
InfA Probe FAM (BHQ-1)	TGCAGTCCTCGCTCACTGGGCACG
Influenza B virus	
InflB Fwd	AAATACGGTGGATTAAATAAAAGCAA
InflB Rev	CCAGCAATAGCTCCGAAGAAA
InflB Probe HEX (BHQ-1)	CACCCATATTGGGCAATTTCCTATGGC
Ribonuclease P	
RP Fwd	AGATTTGGACCTGCGAGCG
RP Rev	GAGCGGCTGTCTCCACAAGT
RP Probe ROX (AbRQSp)	TTCTGACCTGAAGGCTCTGCGCG
Severe acute respiratory syndrome coronavirus 2	
E_Sarbeco_F1	ACAGGTACGTTAATAGTTAATAGCGT
E_Sarbeco_R2	ATATTGCAGCAGTACGCACACA
E_Sarbeco_P1 FAM	ACACTAGCCATCCTTACTGCGCTTCG

3.1.2 PCR reagents and protocol for respiratory virus multiplex RT-qPCR

PCR reagent mix:		
2x PCR Master MIX	12.5 µl	
Primer Mix (5-6 µM primers, 1-2 µM probes)	2.5 µl	
Superscript RT/Platinium Taq	1 µl	
DNA	5 µl	
RNAse free water	adjust to final reaction volume of 25 µl	
PCR program:		
50°C	30 min	45 cycles
94°C	5 min	
94°C	15 sec	
55°C	1 min	

3.1.3 PCR reagents and protocol for SARS-CoV-2 RT-qPCR

PCR reagent mix:		
2x PCR Master MIX	12.5 µl	
E_Sarbeco_F	0.25 µl	
E_Sarbeco_R	0.25 µl	
E_Sarbeco_P	0.5 µl	
RP Fwd	0.25 µl	
RP Rev	0.25 µl	
RP P	0.5 µl	
MgSO4	0.4 µl	
Superscript RT/Platinium Taq	0.5 µl	
RNAse free water	4.6 µl	
DNA	5 µl	
Final reaction volume	**25 µl**	
PCR program:		
55°C	10 min	45 cycles
94°C	3 min	
94°C	15 sec	
58°C	30 sec	

3.1.4 Material and equipment used

Material or equipment	Supplier	Catalog number
PCR primers and probes	Integrated DNA Technologies (IDT), Leuven, Belgium.	
SuperScript™III Platinum® One-Step Quantitative Kit	Invitrogen, Thermo Scientifc Fisher, Waltham, MA, USA	Cat#11732-020 or 11745-100
PCR-grade 8-tubes strips and caps	Invitrogen, Thermo Scientifc Fisher, Waltham, MA, USA	Cat#AM12230
MicroAmp™ Optical 96-Well Reaction Plate	Applied Biosystems, Foster City, CA, USA	Cat# 4306737
1,5 ml reaction tubes	Eppendorf, Hamburg, Germany	Cat#022363204
Table top centrifuge 5425	Eppendorf, Hamburg, Germany	Cat#02231010057
Table top centrifuge 5430 for 96-well plates	Eppendorf, Hamburg, Germany	Cat#022620511
Stratagene Real time PCR Machine (MX 3000P or 3005P)	Agilent Technologies, Santa Clara, CA, USA	Model number: 401513

3.2 SURVEILLANCE DATA COLLECTION AND ANALYSIS

3.2.1 ILI and SARI surveillance in Belgium

Surveillance of ILI was performed every year from week 40 till week 20 through a sentinel system of general practitioners (GP) throughout the country, representing around 1.5% of all Belgian GPs. Patients fulfilling the case definition of acute respiratory infection with onset within the last ten days, with measured fever of ≥38°C and cough and/or dyspnea were eligible to participate in the study. The first two ILI patients belonging to two different households each week were sampled. The ILI surveillance system was active for 32 weeks

each season, except in 2019/2020, where surveillance was interrupted in w13 after 22 weeks due to emergence of SARS-CoV-2 in Belgium.

During the influenza epidemic period (from the last week of December or first/second week of January to the third/last week of April, depending on when influenza virus circulation is detected by the ILI network), SARI surveillance was performed through a network of six sentinel hospitals, two in each administrative region (Flanders, Wallonia and Brussels-Capital). During the study period the weeks of active SARI surveillance in Belgium were as following: 2015/2016: week (w) 01-w17; 2016/2017: w01-w13; 2017/2018: w51-w17; 2018/2019: w01-w15; and 2019/2020: w01-w10. All pediatric and adult patients fulfilling the case definition of acute respiratory infection with onset within the last ten days, with history of fever or measured fever of ≥38°C, with cough and/or dyspnea and overnight hospitalization were eligible for inclusion in the study.

3.2.2 Sample and data collection

Respiratory specimens collected through these networks were sent to the Belgian NIC, along with detailed clinico-epidemiological data, such as information on age, sex, symptoms and potential risk factors such as pregnancy or comorbidities (chronic respiratory diseases, asthma, chronic cardiovascular diseases, renal insufficiency, obesity, diabetes, hepatic or renal insufficiency, immunodeficiency, neuromuscular disease). Hospitalized patients were followed-up for the occurrence of complications (detection of pneumonia based on chest radiography, development of acute respiratory distress syndrome (ARDS), requirement for respiratory assistance and/or for extracorporeal membrane oxygenation (ECMO), admission in intensive care unit (ICU)), or death (all-cause death) during hospitalization.

3.2.3 Determination of the GP and sentinel hospital catchment population

In the GP sentinel network, the catchment population represents the average number of patients that are covered by the GP network per district. To weekly estimate the population covered by the network, the number of inhabitants per active GP (defined as having at least

500 patient contacts/year based on health insurance data) is calculated in each of the 43 districts of Belgium and further multiplied by the number of sentinel practices that participated in each district that week. Only the catchment population of GPs which reported cases to Sciensano were considered per week.

In Belgium, the Federal Public Service provides, the annual market share of hospital admissions of each Belgian general hospital by municipality and age group. The % market share (of registration year – 2) is applied to the each municipalities population and age group. The sum, over all municipalities and sentinel hospitals, provides us with an age-specific catchment population. There are two limitations that may bias the estimate: the market share is estimated on the basis of hospital admissions and not persons. Moreover, only hospitals with a market share of >= 5 % are provided by municipality. The first estimates of this denominator yielded quite plausible catchment populations for each hospital (e.g. in comparison with the number of beds and the regional population structure) and the derived incidences were comparable with estimates based on other (alas equally less than perfect) data sources

3.2.4 Data analysis tools

All data analysis and visualization were performed in R Studio (R version version 3.6.3). The chisq.test and fisher.test function in the stats package, version 4.0.0 were used for statistical analysis. The chisq.test function in R uses a Yates' continuity correction.