Information session for the National Reference Center for Respiratory Pathogens (UZA and UZ/KU Leuven)

26-10-2023

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Recap important information

- **Accreditation** requested: please enter your name (and RIZIV/INAMI if applicable) in the chat box
- **Interactive sessions**: you can speak up by unmuting your microphone to ask questions or raise comments in the chat box

- No recording of the session but slides will be shared
https://www.uzleuven.be/nl/laboratoriumgeneeskunde/nationale-referentiecentra-en-referentielaboratoria
Also on the NRC page on the website of Sciensano

UZ LEUVEN
National Reference Center
Respiratory pathogens

NRC for Respiratory pathogens

sciensano

Nationaal Referentiecentrum (NRC) voor Respiratoire pathogenen

Adenovirus, coronavirus inclusief SARS, human parainfluenza virus, Mycoplasma pneumoniae, Chlamydia pneumoniae, respiratory syncytial virus (RSV), human metapneumovirus (HMPV), Influenza virus

Belangrijke berichten

Algemene informatie

Gelieve de volgende website te contacteren indien u meer informatie wenst over de **wekelijkse rapporten van de SARS-CoV-2 genomic surveillance**: https://www.uzleuven.be/nl/laboratoriumgeneeskunde/genomic-surveillance-sars-cov-2-belgium

Gelieve de volgende website te contacteren indien u meer informatie wenst over de **near real-time wekelijkse detectie van de respiratoire pathogenen in UZ Leuven**: https://www.uzleuven.be/nl/laboratoriumgeneeskunde/wekelijkse-detectieresultaten-respiratoire-pathogenen

Hepatitis — Uitbraak van hepatitis bij kinderen in Europa. (Mei 2022)

Dit event wordt opgevolgd in samenwerking met het NRC Hepatitis

Op 6 april 2022 meldde het Verenigd Koninkrijk (VK) een toename van het aantal gevallen van acute hepatitis bij

Verantwoordelijke laboratoria

Coördinator

- Universitair Ziekenhuis Antwerpen

Geassocieerd

- UZ Leuven/KU Leuven

Erkend door

- National Institute for Health and Disability Insurance (INAMI-RIZIV)

Aanvraagformulieren

- Aanvraagformulier respiratoire pathogenen

Complementarity of two laboratories

UZ Antwerp: respiratory bacteria

- PCR *M. pneumoniae* and *C. pneumoniae*
- PCR macrolide resistance for *M. pneumoniae*
- PCR respiratory viruses in the context of outbreaks (broad respiratory panel)

UZ Leuven: respiratory viruses

- Detection and evolution of coronaviruses (incl. SARS-CoV-1/2 and MERS)
- Molecular typing of RSV
- PCR respiratory viruses in the context of outbreaks (broad respiratory panel)

Epidemiological waves and macrolide resistance in *Mycoplasma pneumoniae*

Mycoplasma pneumoniae

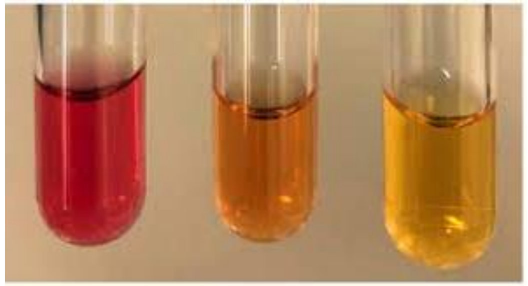
Introduction → bacterium

- Member of the *Mollicutes* class (“mollis” = soft, “cutis” = skin) → mucosal pathogens

- Smallest self-replicating organisms capable of cell-free existence
  - Cellular dimension (spindle-shaped: 1-2 µm long, 0.1-0.2 µm wide)
  - Genome size (0.8 Mbp) → reduced and highly stable

- Lacks a cell wall → resistant to cell wall synthesis inhibitors

- Can be cultured
  - Grows slowly: generation time 6h → incubation up to 3 weeks – 2 months
  - Enriched broth or agar (SP4) → limited biosynthetic capacities
  - “fried egg” colony morphology → visualized microscopically



Mycoplasma pneumoniae

Introduction → pathogenesis

- Transmission via droplets
- Incubation period: 4 days -3 weeks
- Respiratory tract infections mostly mild and self-limiting
  - “walking pneumonia” ←→ Severe CAP
  - Atypical pneumonia:
    - fever, sore throat, (typically non-productive) cough, chest pain, absence of wheeze
    - Rx: bilateral, diffuse interstitial infiltrates common, pleural effusion can occur
    - persistence of cough for weeks to months
  - Extrapulmonary manifestations: skin manifestations, encephalitis, Guillain-Barré
    - Direct local effects of *M. pneumoniae* after dissemination
    - Indirect immune-mediated effects

Table 1. Pulmonary and Extrapulmonary Manifestations of *Mycoplasma pneumoniae* Infection

Organ involvement	Manifestation
Pulmonary	Asthma/chronic obstructive pulmonary disease (COPD) exacerbation Tracheobronchitis Pneumonia: lobar and multi-lobar infiltrates Diffuse alveolar hemorrhage
Gastrointestinal	Nausea, vomiting, abdominal pain, anorexia Diarrhea Transaminitis
Cardiovascular	Myocarditis, pericarditis Cardiac arrhythmias Thrombotic events
Neurological	Meningitis, encephalitis, optic neuritis Guillain-Barre syndrome
Renal	Acute tubular necrosis, glomerulonephritis, interstitial nephritis
Musculoskeletal/skin	Erythema nodosum, cutaneous leukocytoclastic vasculitis Erythema multiforme, Stevens-Johnson syndrome MP-associated mucositis Myopathy, arthritis, and rhabdomyolysis
Thrombotic	Pulmonary embolism Splenic artery and left atrium and right ventricle thrombosis Aortic thrombosis/renal artery thrombosis
Other	Vasculitis (positive antineutrophil cytoplasmic antibodies) Cytopenias, cold agglutinin-induced autoimmune hemolytic anemia, sickle cell disease, idiopathic thrombocytopenic purpura-like syndrome Kawasaki disease

Bajantri B et al. *Mycoplasma pneumoniae*: A Potentially Severe Infection. J Clin Med Res 2018;10(7):535–44.

Mycoplasma pneumoniae

Introduction → epidemiology and treatment

- Endemic worldwide (many different climates)
- Infection throughout the year → seasonal variation (temperate climates: peak during latter months/year)
- Epidemic waves/peaks every 4-7 years: last wave April 2019 – March 2020

- Affects all ages → most frequent among school-age children 5-15y and young adults

- Treatment: macrolides, tetracyclines and fluoroquinolones → IGGI guideline:

- ***Gerichte anti-infectieuze behandeling***
  - Patiënten zonder IgE gemedieerde allergie voor penicillines.
    - Pasgeborenen: **azithromycine of clarithromycine**.
    - Zuigelingen, kinderen < 8 jaar.
      - Eerste keuzes: **azithromycine of clarithromycine**.
      - Tweede keuze: **ciprofloxacine**[1].
    - Kinderen ≥ 8 jaar.
      - Eerste keuzes: **azithromycine of clarithromycine**.
      - Tweede keuzes: **ciprofloxacine**[1] **of doxycycline**[2].
    - Adolescenten.
      - Eerste keuzes: **azithromycine of clarithromycine**.
      - Tweede keuzes: **ciprofloxacine**[1] **of doxycycline**[2] **of levofloxacine**[3].
    - Volwassenen.
      - Eerste keuzes: **azithromycine of clarithromycine**.
      - Tweede keuzes: **ciprofloxacine**[1] **of doxycycline**[2] **of levofloxacine**[3] **of moxifloxacine**[4].
    - Zwangere[5] patiënten: **azithromycine of clarithromycine**.
    - Borstvoedende[5] patiënten: zoals bij adolescenten of volwassenen + (tijdelijke) stopzetting van de borstvoeding tijdens de anti-infectieuze behandeling indien ciprofloxacine[1], doxycycline[2], levofloxacine[3] of moxifloxacine[4] gebruikt wordt.

Mycoplasma pneumoniae

Detection techniques

- PCR = gold standard

- Serology
    - IgM within 1 week after infection
    - Sensitivity depends on time point 1st sample and availability of paired serum samples ≥ 2 weeks

- Antigen test: less sensitive → detection limit $1x10^3$ CFU/ml

- Culture: sensitivity 60%, specificity 100%

Mycoplasma pneumoniae

Global incidence

- Study by ESGMAC (ESCMID Study Group for Mycoplasma and Chlamydia Infections)

  → the effect of non-pharmaceutical interventions (NPIs) against COVID-19 on transmission of *M. pneumoniae*

    - Physical distancing
    - Personal protective measures
    - Stay-at-home orders
    - School and daycare closure
    - Closing of borders, travel restrictions

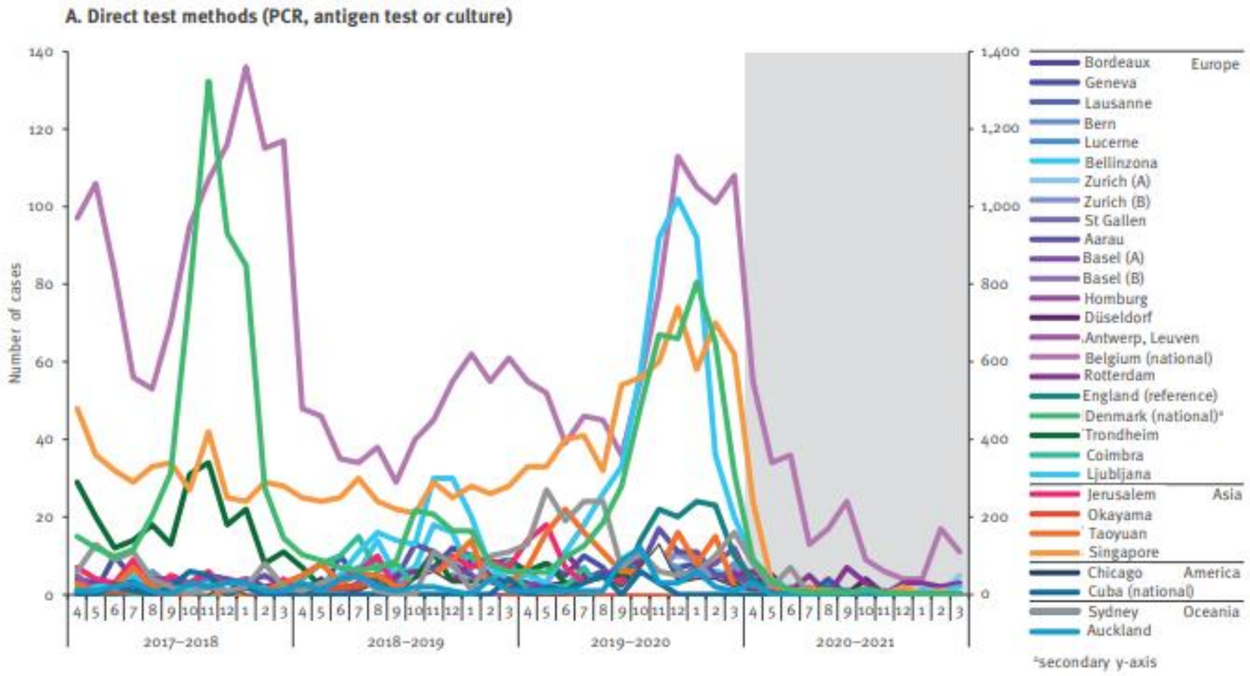
Meyer Sauteur PM et al. *Mycoplasma pneumoniae* detections before and during the COVID-19 pandemic: results of a global survey, 2017 to 2021. Eurosurveillance 2022;27(19):pii=2100746

Mycoplasma pneumoniae

Global incidence

- Pre-COVID (2017-2020) → incidence **8.61%**
- During COVID (2020-2021) → incidence **1.69%** → also lower transmission of other respiratory pathogens

Global detection of *Mycoplasma pneumoniae*, April 2017–March 2021 (n = 30,617)

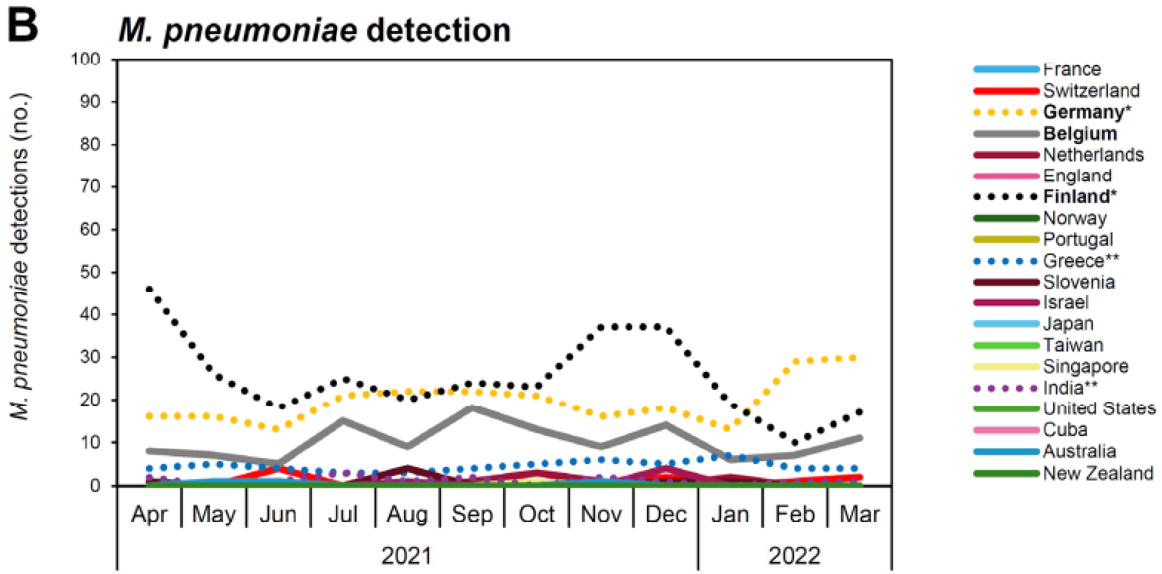


Meyer Sauteur PM et al. *Mycoplasma pneumoniae* detections before and during the COVID-19 pandemic: results of a global survey, 2017 to 2021. Eurosurveillance 2022;27(19):pii=2100746

Mycoplasma pneumoniae

Global incidence

- Pre-COVID (2017-2020) → incidence **8.61%**
- During COVID (2020-2021) → incidence **1.69%**
- During-after COVID (April 2021- March 2022) → incidence **0.70%** → relaxed or discontinued NPI's → resurge of other respiratory pathogens (increased community transmission)

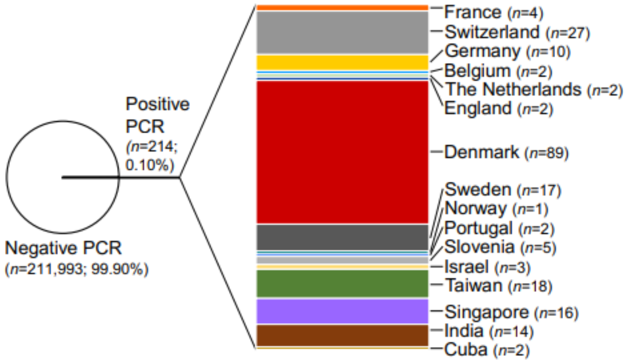


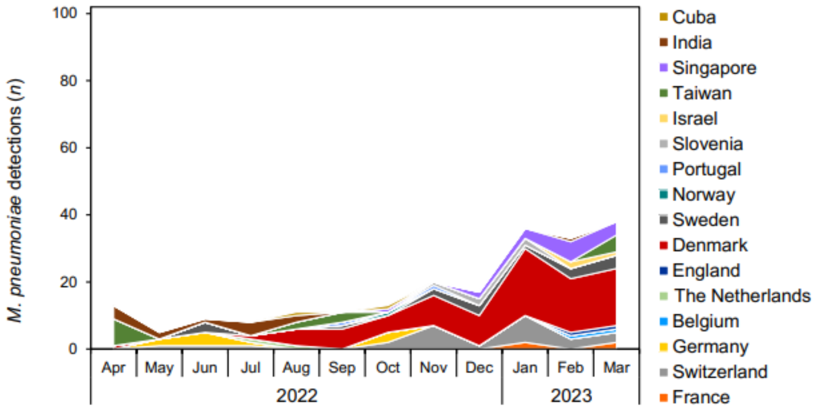
Meyer Sauteur PM et al. *Mycoplasma pneumoniae:* beyond the COVID-19 pandemic: where is it?. Lancet Microbe 2022;3:e897

Mycoplasma pneumoniae

Global incidence

- Pre-COVID (2017-2020) → incidence **8.61%**
- During COVID (2020-2021) → incidence **1.69%**
- During-after COVID (April 2021- March 2022) → incidence **0.70%**
- April 2022 – March 2023 → incidence **0.82%**





Why?

- Direct effect of SARS-CoV-2 on *M. pneumoniae*?
- Herd immunity from the last *M. pneumoniae* wave (2019-2020)?

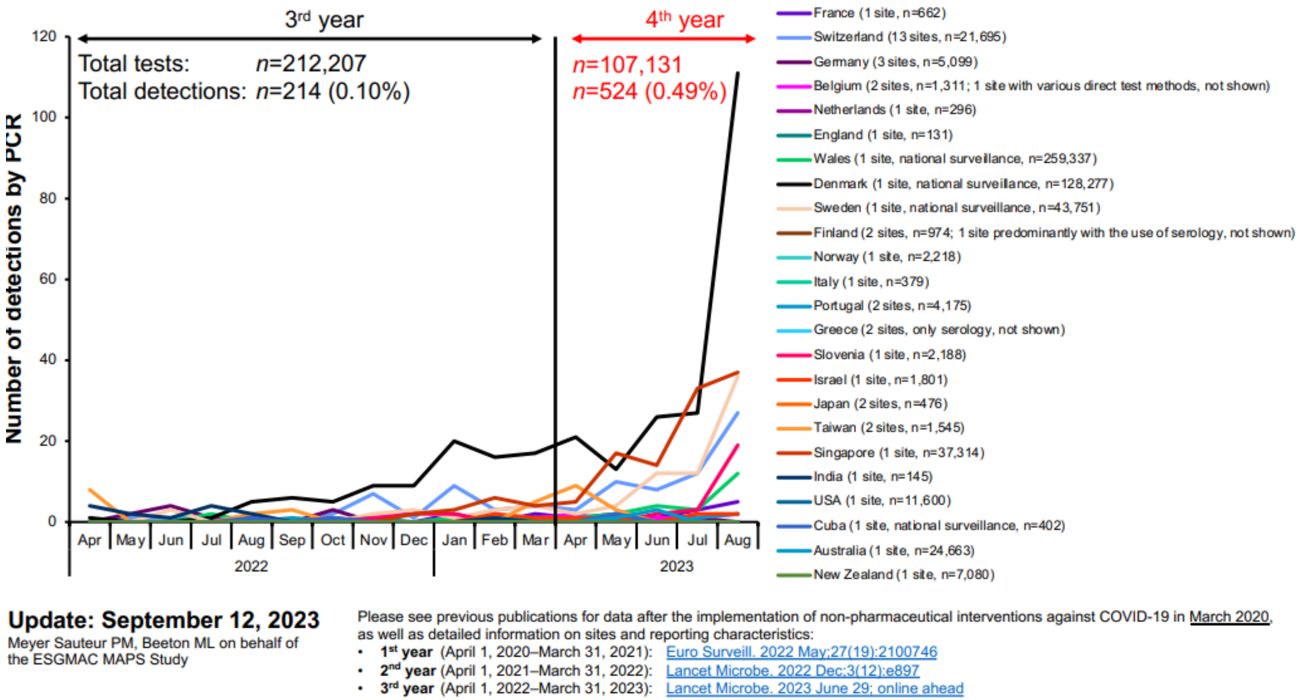
→ Gone forever?

Meyer Sauteur PM et al. *Mycoplasma pneumoniae:* gone forever?. Lancet Microbe 2023;4(10):e763

Mycoplasma pneumoniae

Global incidence

- Ongoing resurgence
- ESCMID: ESGMAC MAPS study



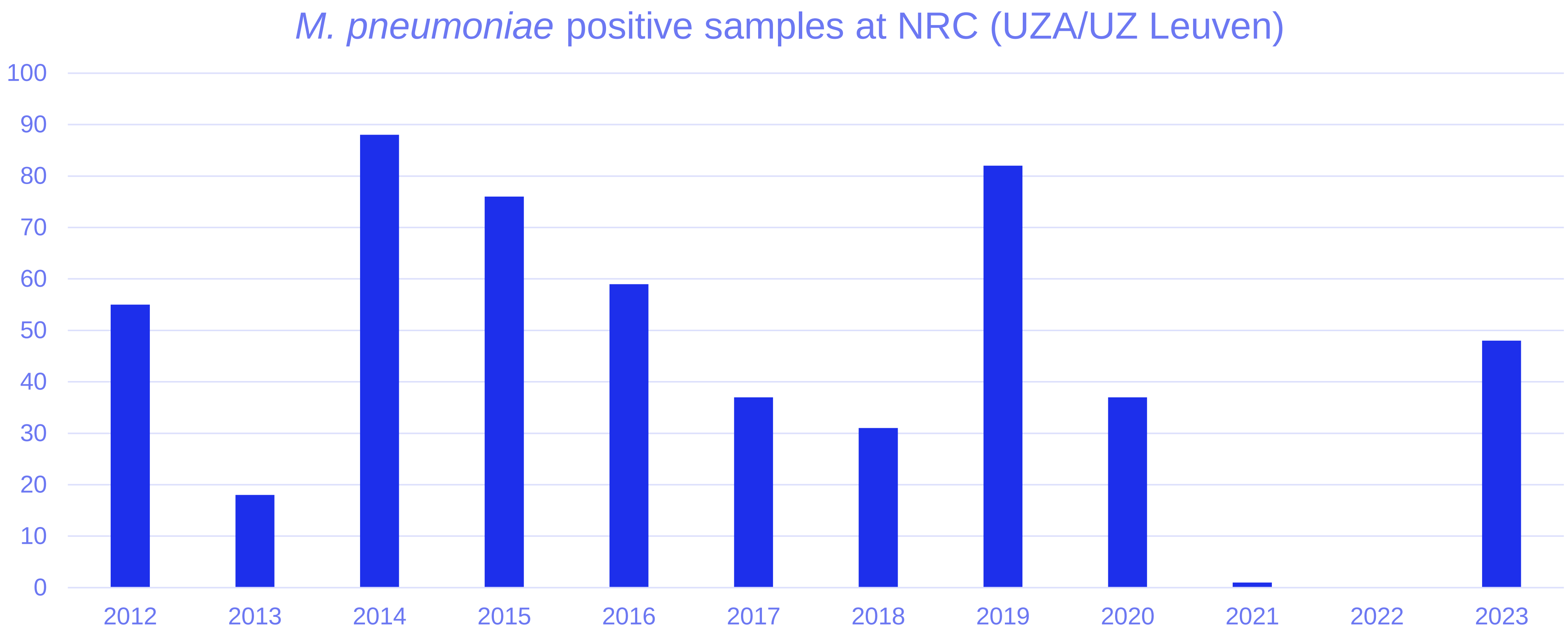
- Resurgence in a population not exposed to *M. pneumoniae* for 3 years

→ rare severe disease and extrapulmonary manifestations?

Mycoplasma pneumoniae

Belgian data

NRC data (combination of samples received at UZ Leuven and UZA)



Mycoplasma pneumoniae

Macrolide resistance → rates

2003-2015

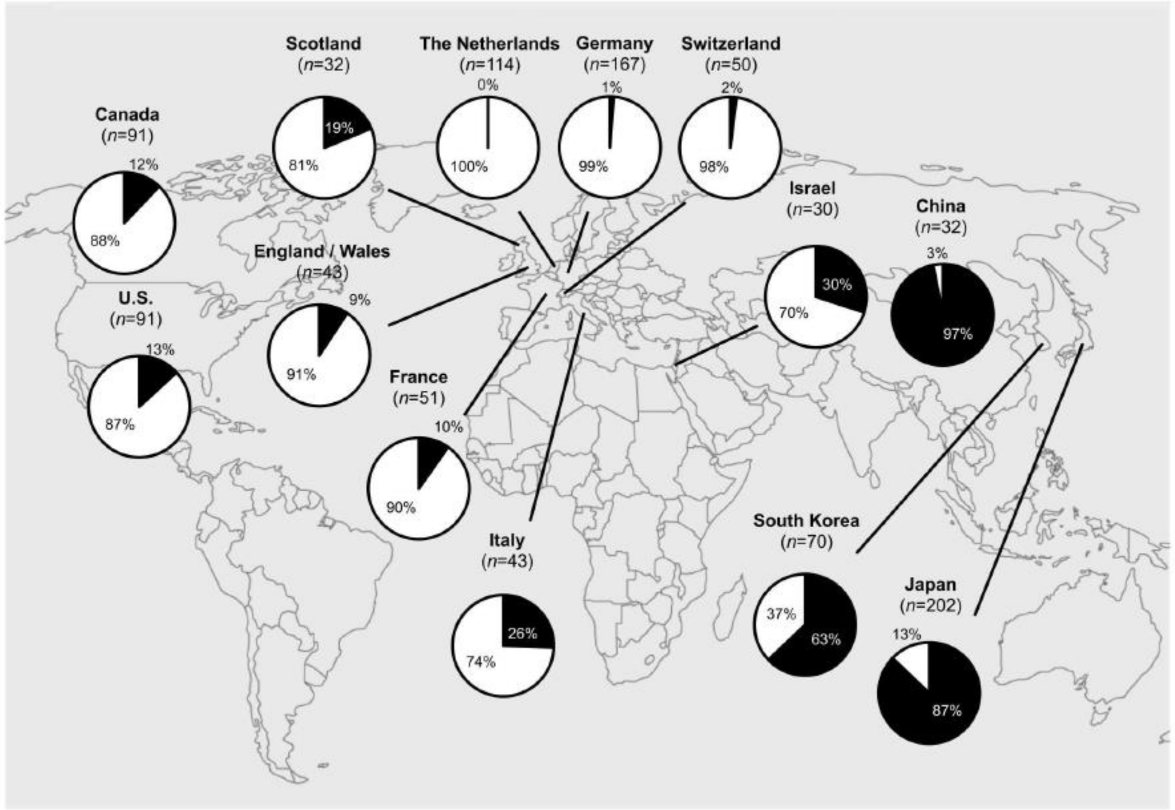


FIGURE 6 | Worldwide macrolide-resistant *M. pneumoniae* (MRMP) rates. Actual MRMP rates are punctually depicted in pie charts (in black) over the world map. **Asia:** Japan (2011): 87% (176/202) (Okada et al., 2012), South Korea (2011): 63% (44/70) (Hong et al., 2013), China (2012): 97% (31/32) (Zhao et al., 2013), Israel (2010): 30% (9/30) (Averbuch et al., 2011); **North America:** U.S. (2012–2014): 13% (12/91) (Zheng et al., 2015), Canada (2010–2012): 12% (11/91) (Eshaghi et al., 2013); **Europe:** The Netherlands (1997–2008): 0% (0/114) (Spuesens et al., 2012), Germany (2003–2008): 1% (2/167) (Dumke et al., 2010), France (2005–2007): 10% (5/51) (Peuchant et al., 2009), Italy (2010): 26% (11/43) (Chironna et al., 2011), Scotland (2010–2011): 19% (6/32) (Ferguson et al., 2013), Switzerland (2011–2013): 2% (1/50) (Meyer Sauteur et al., 2014a), England and Wales (2014–2015): 9% (4/43) (Brown et al., 2015).

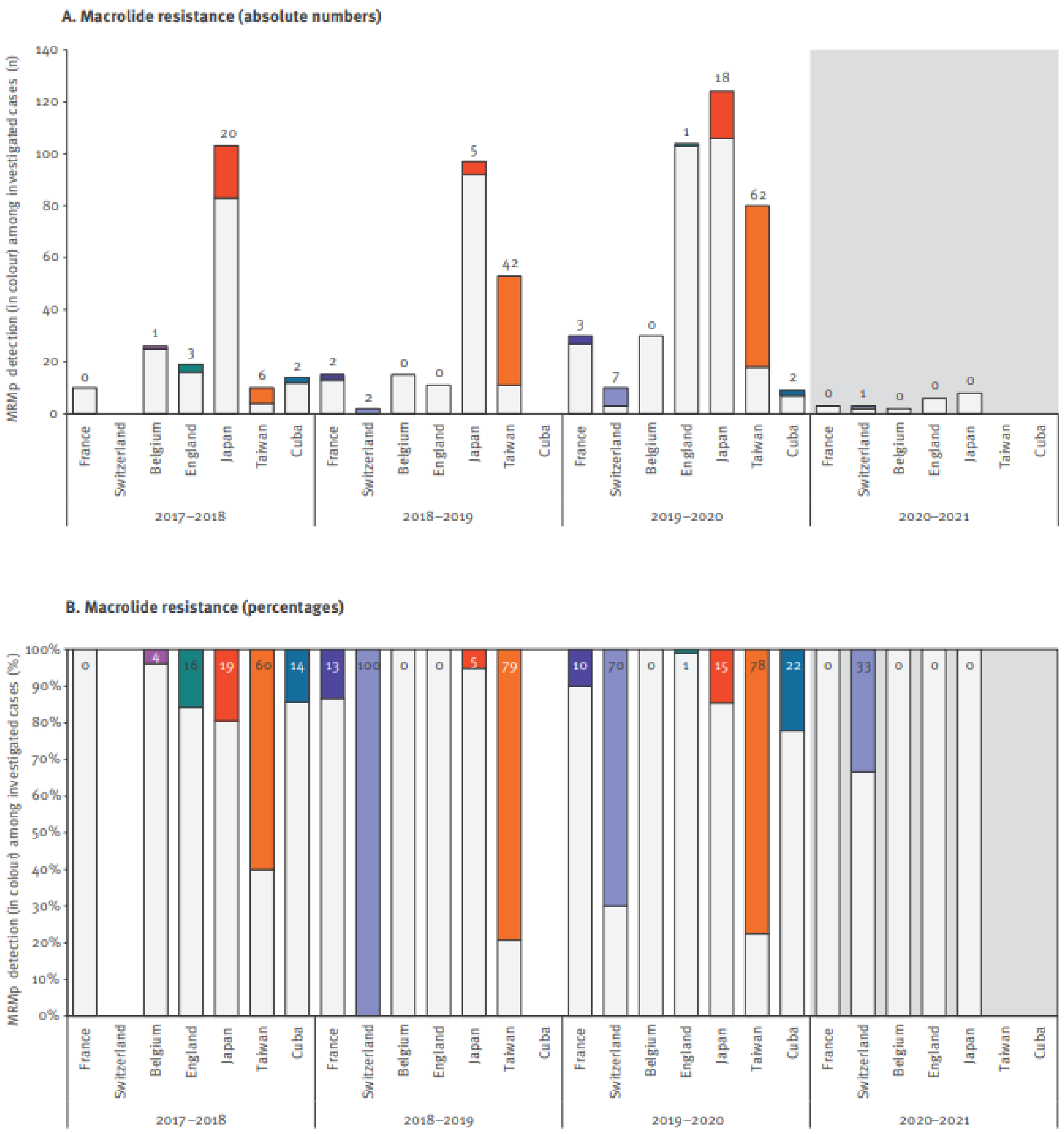
Meyer Sauteur PM et al. Infection with and Carriage of *Mycoplasma pneumoniae* in Children. Front Microbiol. 2016;7:329

Mycoplasma pneumoniae

Macrolide resistance → rates

2017-2021

Macrolide-resistant *Mycoplasma pneumoniae* testing and detection in different countries across the world, April 2017–March 2021 (n = 784)



Meyer Sauteur PM et al. *Mycoplasma pneumoniae* detections before and during the COVID-19 pandemic: results of a global survey, 2017 to 2021. Eurosurveillance 2022:27(19):2100746

Mycoplasma pneumoniae

Macrolide resistance → mutations and techniques

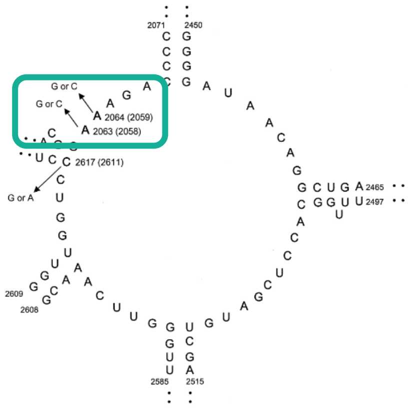
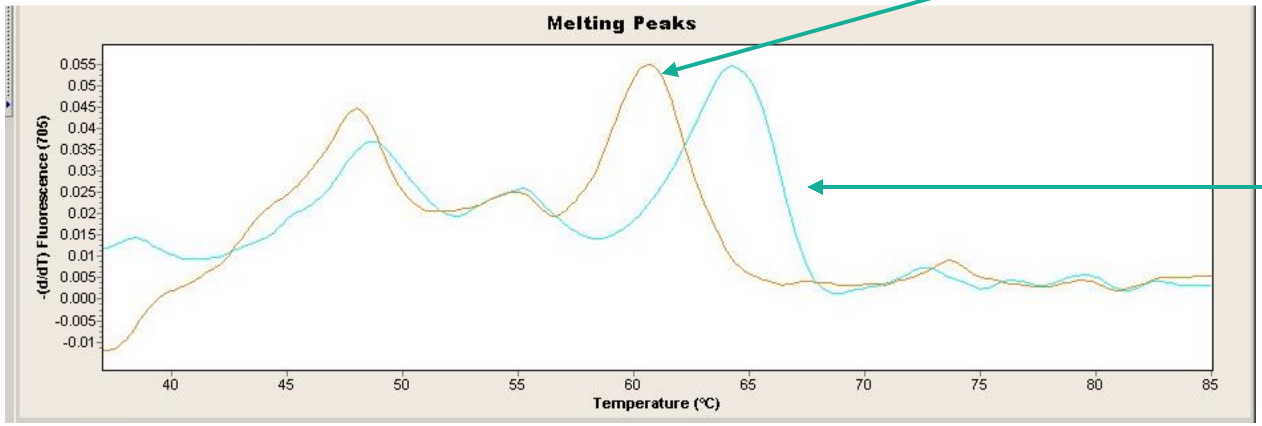


FIG. 2. Secondary structure of the peptidyltransferase loop in domain V of *M. pneumoniae* 23S rRNA. Positions of the newly found mutations (A2063C and C2617G), as well as previously reported in vitro mutations (A2063G, A2064G, and A2064C), in clinical isolates are indicated by using the numbering for *M. pneumoniae* 23S rRNA (accession no. X68422). The numbers in parentheses indicate *E. coli* numbering.

- Point mutation in the peptidyl transferase loop of domain V of the 23S rRNA gene
  - → Mostly A- to G- transitions (A2063G, A2064G, A2063T)
  - → Reduced affinity of the macrolide for the ribosomes
- Duplex real-time PCR with melting curve analysis

Macrolide mutation Tm 60.5



Wildtype Tm 64.5

- Less sensitive than *M. pneumoniae* PCR (P1 target: 8-10 copies/genome ←→ 23S rRNA target: 1 copy/genome)

Peuchant O, Ménard A, Renaudin H, Morozumi M, Ubukata K, Bébéar CM, et al. Increased macrolide resistance of *Mycoplasma pneumoniae* in France directly detected in clinical specimens by real-time PCR and melting curve analysis. Journal of Antimicrobial Chemotherapy 2009; 64: 52-58

Mycoplasma pneumoniae

Macrolide resistance

- Clinical impact
  - Children admitted to Chinese University Hospital with *M. pneumoniae* pneumonia

199 A2063G → 97%
6 A2063T → 3%
1 A2064G → < 1%

TABLE 1 Clinical information of MR and MS patients

Clinical information	MR group (*n* = 206)	MS group (*n* = 29)	*P*
Median age in yrs (range)	4 (0–14)	5 (0–11)	NS[b]
No. of patients (male/female)	132/74	17/12	NS
% Patients with severe MPP	18.4 (38/206)	3.4 (1/29)	0.042
% Patients with large lesions on chest radiography[a]	61.7 (127/206)	41.3 (12/29)	0.038
Median durations in days (range)			
Fever duration	8 (0–42)	6 (0–14)	0.001
Hospitalization duration	8 (2–45)	6 (3–16)	0.007
Fever duration after macrolide therapy	5 (0–42)	3 (0–10)	0.007
% Patients presenting with extrapulmonary complications	29.6 (61/206)	10.3 (3/29)	0.029
Digestive system (liver function abnormalities)	44.3 (27/61)	100 (3/3)	
Cardiovascular system (myocarditis)	24.6 (15/61)	0 (0/3)	
Rash	18.0 (11/61)	0 (0/3)	
Nervous system (encephalitis)	6.6 (4/61)	0 (0/3)	
Urinary system (proteinuria)	3.3 (2/61)	0 (0/3)	
Hematologic system (hemolytic anemia)	1.6 (1/61)	0 (0/3)	
Joint system (arthritis)	1.6 (1/61)	0 (0/3)	

[a] A large lesion was determined when the extent of infiltration on chest radiography was more than one-third of the lung.
[b] NS, not significant.

Zhou Y, Zhang Y, Sheng Y, Zhang L, Shen Z, Chen Z. More complications occur in macrolide-resistant than in macrolide-sensitive Mycoplasma pneumoniae pneumonia. Antimicrobial agents and chemotherapy 2014;58(2):1034-8.

Mycoplasma pneumoniae

Macrolide resistance

- Therapeutic options → tetracyclines (minocycline) or fluoroquinolones?

in vitro

Table 2. ***In vitro*** **anti-mycoplasma activities against clinical isolates of** ***M. pneumoniae*** **with or without A2063G mutation in the 23S rRNA gene.**

Antimicrobial agent	MIC (μg/ml) for MRMP (n = 27)			MIC (μg/ml) for MSMP (n = 23)		
	Range	50%	90%	Range	50%	90%
Erythromycin	128 - >256	256	>256	0.002–0.0078	0.0039	0.0039
Clarithromycin	64 - >256	256	256	0.0005–0.0039	0.001	0.001
Azithromycin	16–128	32	64	<0.000125–0.00025	<0.000125	<0.000125
Clindamycin	16–256	64	128	0.13–0.5	0.25	0.5
Levofloxacin	0.25–0.5	0.5	0.5	0.25–0.5	0.5	0.5
Ciprofloxacin	0.5–1	1	1	0.5–2	1	1
Tosufloxacin	0.13–0.25	0.25	0.25	0.13–0.5	0.25	0.5
Minocycline	0.13–1	0.5	1	0.13–2	0.5	1

MRMP, macrolide-resistant *Mycoplasma pneumoniae*; MSMP, macrolide-sensitive *Mycoplasma pneumoniae*.

in vivo

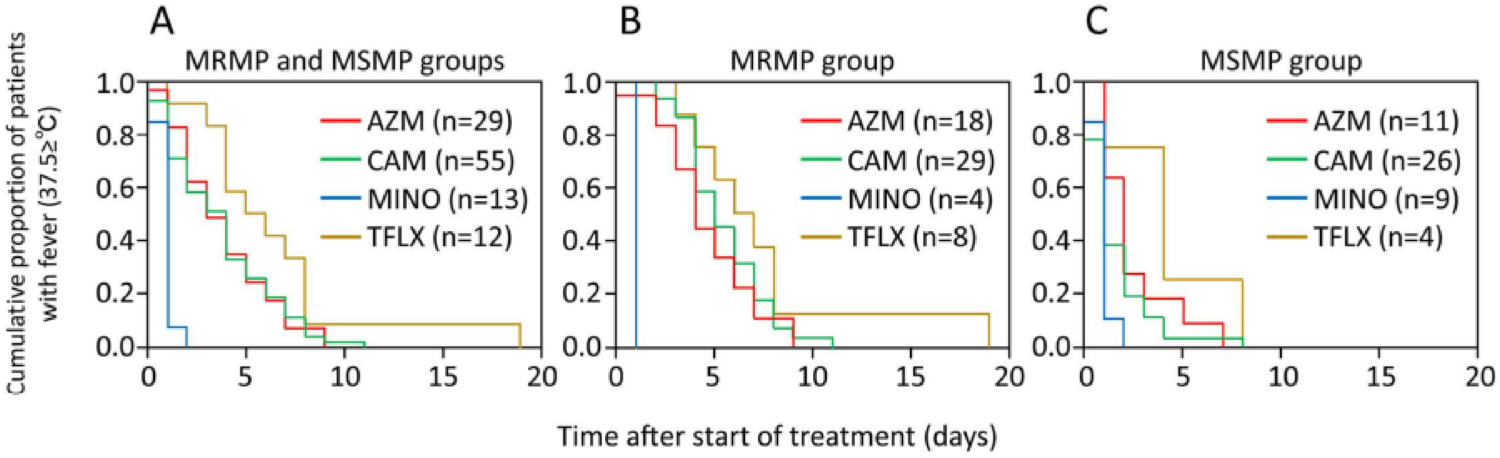


Fig 1. Durations of fever following commencement of treatment for pneumonia due to MRMP and MSMP by azithromycin, clarithromycin, minocycline and tosufloxacin. Kaplan–Meier curves showing a comparison of times taken for body temperature to return to <37.5˚C among patients with (A) MRMP and MSMP (log-rank test, $P < 0.0001$), (B) MRMP (log-rank test, $P < 0.0001$) and (C) MSMP (log-rank test, $P = 0.0162$).

Duration of fever (days)

	MRMP	MSMP	p-value
azithromycin	4.6	2.5	0.0175
clarithromycin	5.4	1.7	<0.0001
minocycline	1.0	0.9	0.7496
tosufloxacin	7.5	4.3	0.3166

Ishiguro N et al. Therapeutic efficacy of azithromycin, clarithromycin, minocycline and tosufloxacin against macrolide-resistant and macrolide-sensitive *M. pneumoniae* pneumonia in paediatric patients. Plos One 2017;12(3):e0173635.

Mycoplasma pneumoniae

Macrolide resistance → Belgium

	Macrolide resistant (n)	*M. pneumoniae* positives (n)	% resistance
2012	1	6	17
2013	0	11	0
2014	4	68	6
2015	3	62	5
2016	2	36	6
2017	0	32	0
2018	1	15	7
2019	0	22	0
2020	1	32	3
2021	0	0	/
2022	0	0	/
2023	0	18	0

All A2063G

NRC request form

UZA

REFERENTIECENTRUM VOOR RESPIRATOIRE PATHOGENEN

GELIEVE HET STAAL SAMEN MET DIT INGEVULD FORMULIER OP TE STUREN NAAR:
Dr. V. Matheeussen
Universitair Ziekenhuis Antwerpen - Microbiologie
Drie Eikenstraat 655, 2650 Edegem
Tel 03/821 36 67, Fax: 03/821 38 74, email: referentiecentrum@uza.be

***GEGEVENS OVER HET LABORATORIUM DAT HET STAAL OPSTUURT**

Naam klinisch bioloog: ……………………………
Naam laboratorium: ……………………………
Tel: ………………… Fax: ……………………
Emailadres: ……………………………………
Naam+ RIZIVnr aanvragende arts: ……………………
……………………………………………………

***PATIENTGEGEVENS OF STICKER**

Naam: ……………………………………………
Geslacht: ❑ M ❑ V
Geboortedatum: …………………………………
Rijksregisternr: …………………………………
Straat+nr: ………………………………………
Postcode of woonplaats: …………………………
Nationaliteit: ……………………………………
Recent verblijf buitenland: ❑ ja ❑ neen
Zo ja, land of streek: ……………………………

***GEGEVENS OVER HET STAAL**

Identificatienummer: ……………………………
Afnamedatum: …………………………………
❑ keelwisser ❑ sputum
❑ biopt (niet gefixeerd) ❑ BAL
❑ nasopharyngeale (flocked) swab
❑ nasopharyngeaal aspiraat
❑ CSV (*M. pneumoniae*) eiwitgehalte……….mg/dl
%lymphocyten …….. glucosegehalte…….mg/dl
Aantal cellen ❑0-5 ❑6-10 ❑11-49 ❑>50
❑ conjunctivale wisser (adenovirus)
❑ andere ……………………………………
❑ Resultaat Gram kleuring ……………………
❑ Resultaat kweek ……………………………

ANDERE BELANGRIJKE GEGEVENS

Indien van toepassing, bvb mogelijke urgentie van analyse

***KLINISCHE GEGEVENS**

Datum begin symptomen:……………………………
Antibioticabehandeling voorbije 48h ❑ ja ❑ nee ❑ onbekend
als ja welk……………………………………………
Hospitalisatie ❑ ja ❑ nee
als ja : ❑ infectieziekten ❑ intensieve zorgen
❑ spoedgevallen ❑ pediatrie
❑ andere……..
RX pneumonie: ❑ ja ❑ nee ❑ onbekend
Koorts ❑ ja ❑ nee
Hoest ❑ ja ❑ nee
Conjunctivitis ❑ ja ❑ nee
Kortademig ❑ ja ❑ nee
Onderliggend longlijden ❑ ja preciseer………… ❑ nee
Immunodeficiënt ❑ ja preciseer………… ❑ nee
Hoofdpijn ❑ ja ❑ nee
Spierpijn ❑ ja ❑ nee
Meningitis ❑ ja ❑ nee
Encephalitis ❑ ja ❑ nee

Vermoeden van uitbraak: ❑ ja ❑ nee
❑ Index ❑ contactgeval
Naam index ………………………
Relatie tot index……………………
Contact opgenomen met de arts infectieziektebestrijding uit uw provincie of regio: ❑ ja ❑ nee

AANGEVRAAGDE TESTEN NRC

❑ PCR *M. pneumoniae* macrolide resistentie (bij positieve PCR)
❑ PCR *C. pneumoniae* (indien negatief voor *M. pneumoniae, S. pneumoniae*, influenza, RSV én patiënt gehospitaliseerd is met een lage luchtweginfectie)
❑ PCR respiratoire virussen (enkel in geval van uitbraak, inclusief SARS, MERS en COVID-19)

***Verplicht in te vullen** **Aanvraagformulier NRC Respiratoire pathogenen NL 210727**

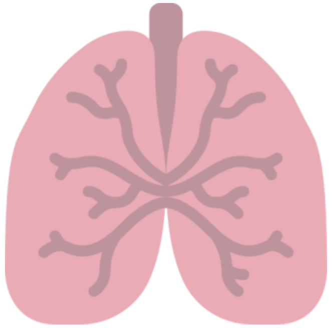
- Ct value of PCR *M. pneumoniae* < 35
- Minimal volume
  - Sample: 250 µl
  - Extract: 20 µl

Epidemiology and molecular typing of respiratory syncytial virus (RSV)

UZ LEUVEN
National Reference Center
Respiratory pathogens

Detection of respiratory pathogens

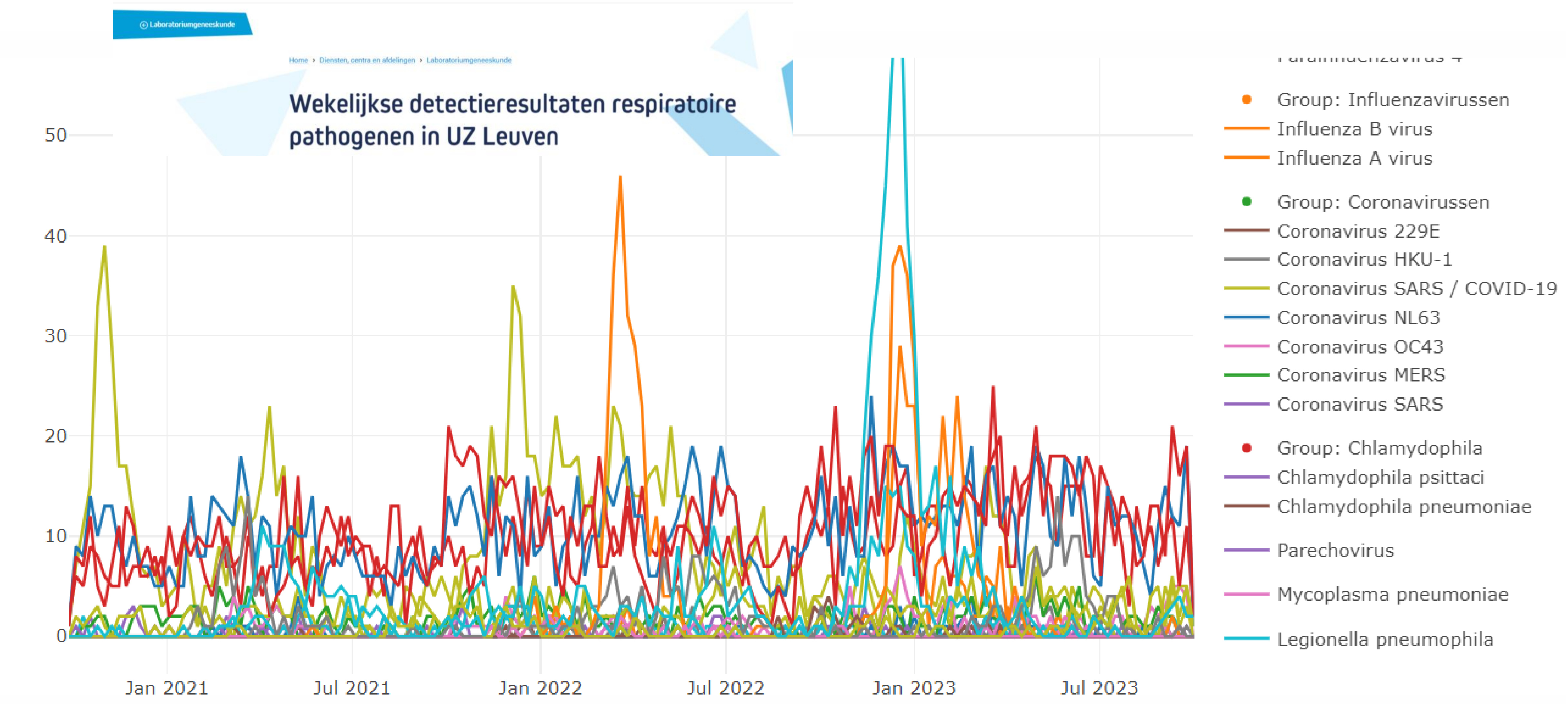
- **Broad respiratory panel**: 29 parameters
    - 22 viruses, 1 fungus and 6 bacteria
    - Oro/nasopharyngeal swabs, aspirates and BAL
    - Semi-quantitative reporting
    - LDT
    - Implementation 2016:
        - *S. pneumoniae* since 2017
        - SARS-CoV-2 since 2020
    - Nomenclature respiratory pathogens – NRC – 75€



Volgende virussen, bacteriën en een fungus worden gedetecteerd:

- influenza A/B virus
- respiratoir syncitiaal virus (RSV)
- humaan metapneumovirus (hMPV)
- parainfluenza virus 1/2/3/4
- adenovirus
- enterovirus / rhinovirus
- cytomegalovirus
- parechovirus
- coronavirus NL63 / 229E / OC43 / HKU-1
- coronavirus SARS inclusief SARS-CoV-2
- coronavirus MERS
- herpes simplex virus 1/2
- bocavirus
- Mycoplasma pneumoniae
- Pneumocystis jirovecii
- Coxiella burnetti
- Chlamydophila pneumoniae
- Chlamydophila psittaci
- Legionella pneumophila
- Streptococcus pneumoniae

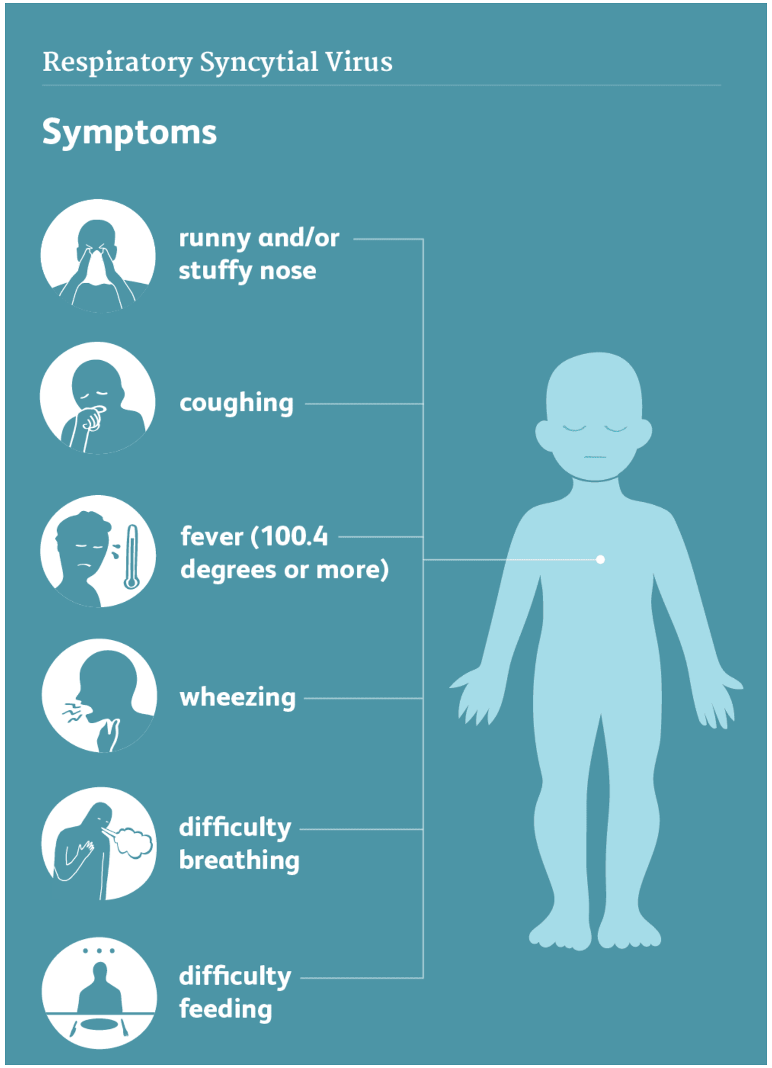
Epidemiology of respiratory pathogens



Detection of human respiratory syncytial virus (HRSV)

- Broad respiratory panel (‘**maxi-panel**’): among the 29 parameters (importance and frequency of co-infections)
  ~ **SARI** (severe acute respiratory infection)

- **Respiratory mini-panel**: 4 parameters ~ **ILI** (influenza-like illness)
  - SARS-CoV-2, RSV, Influenza A and Influenza B
  - Semi-quantitative reporting
  - Continu analyser Alinity M and urgent PCR GeneXpert
  - Implementation 2022
  - Before and early COVID: influenza/RSV PCR (Panther): €25
  - Nomenclature respiratory pathogens



From https://www.verywellhealth.com/rsv-symptoms-treatments-770635

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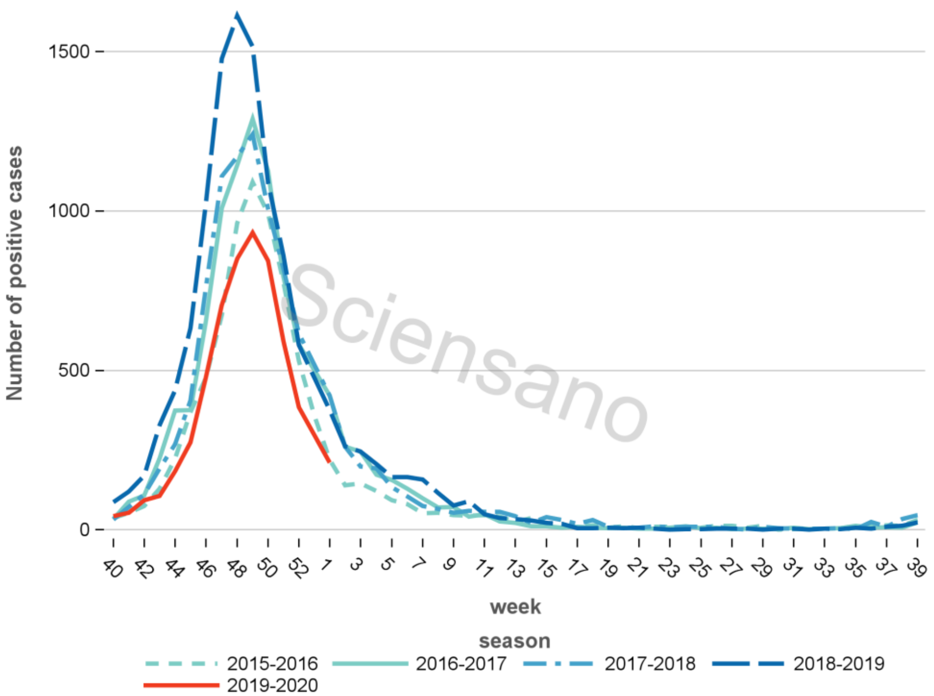
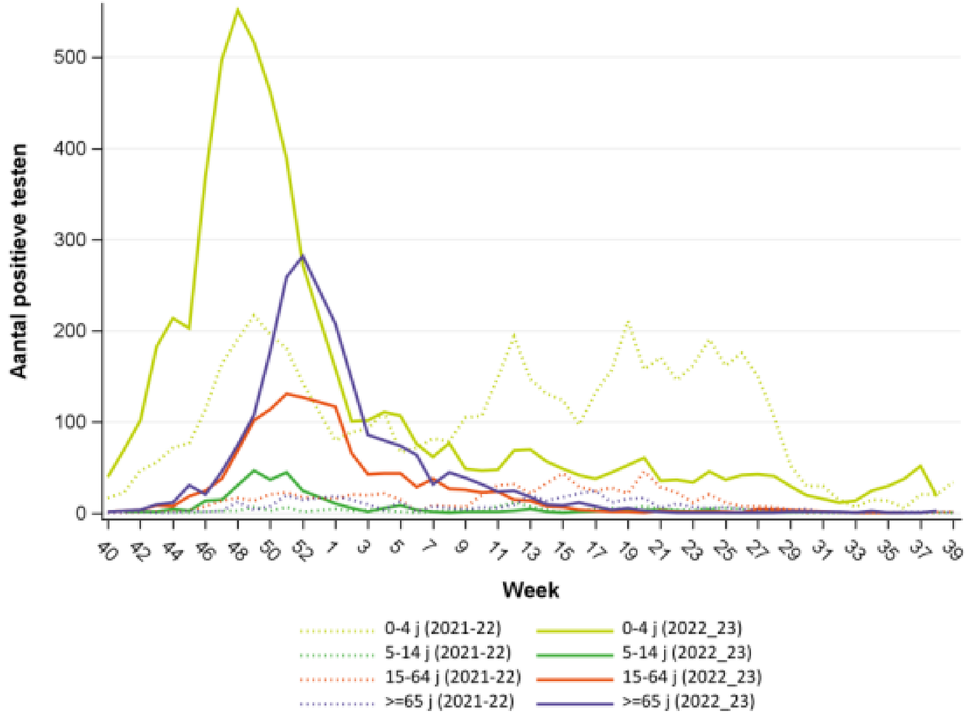
Epidemiology of RSV

- **Most common respiratory infection for neonates and very young children**
- Transmission via droplet infection and (in)direct contact
- Every year >7000 infections reported through the sentinel laboratory network





From respiratory bulletin Sciensano

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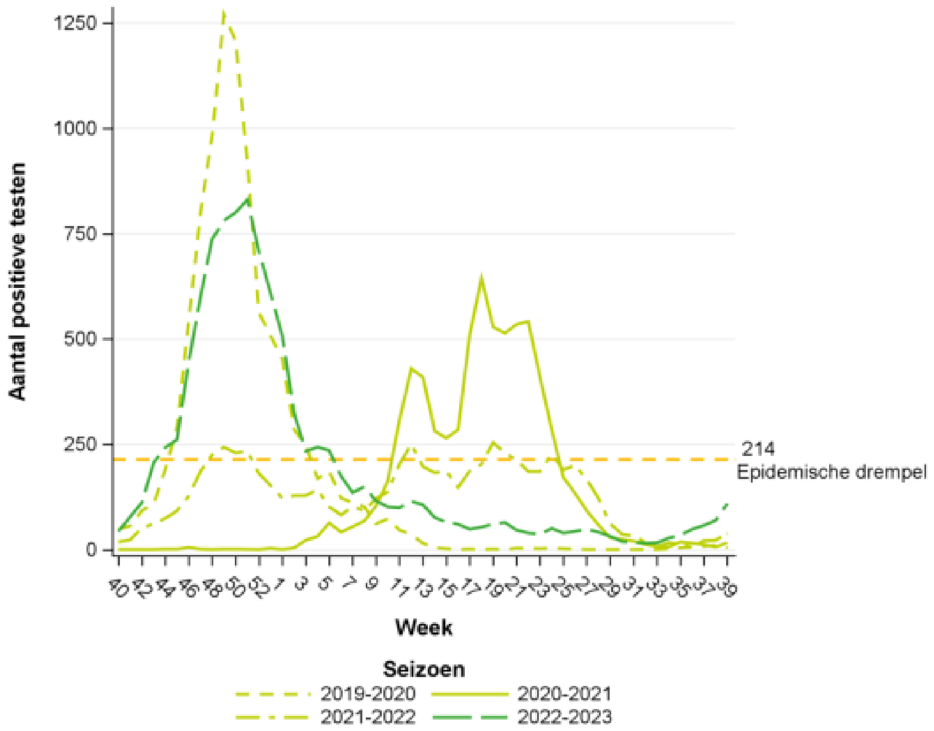
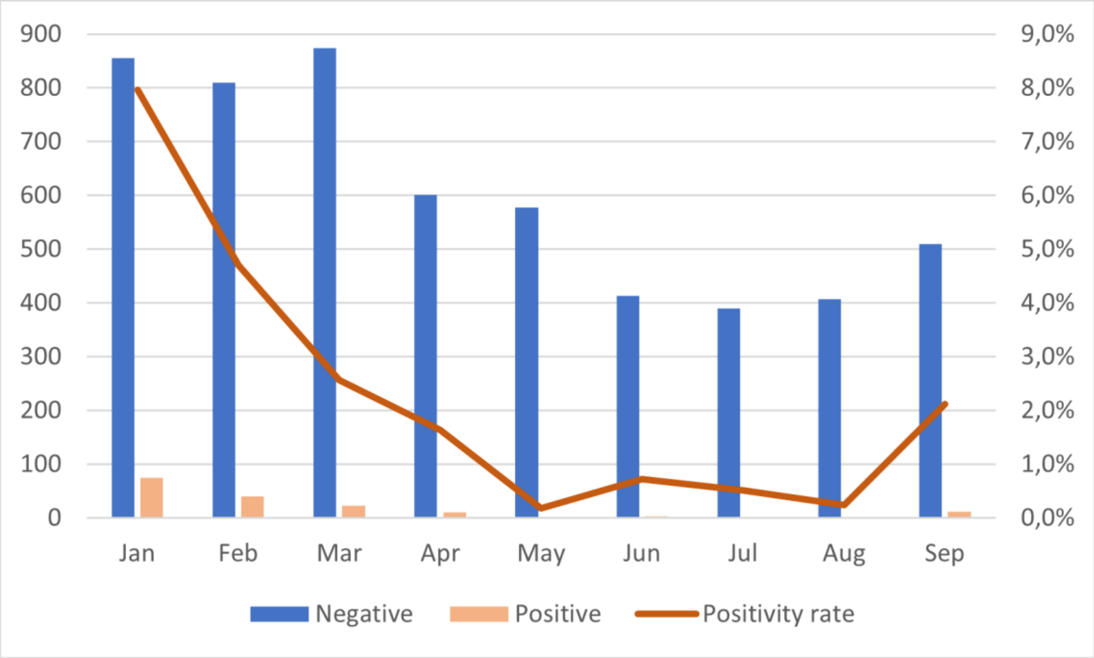
Respiratory pathogens

Seasonality of RSV

- General RSV season = **start October to end March** (peak half December)
- **Disturbed during the COVID-19 pandemic**: very late 2020-2021 season
- Current season 2023-2024 started early: first cases in September





From respiratory bulletin Sciensano

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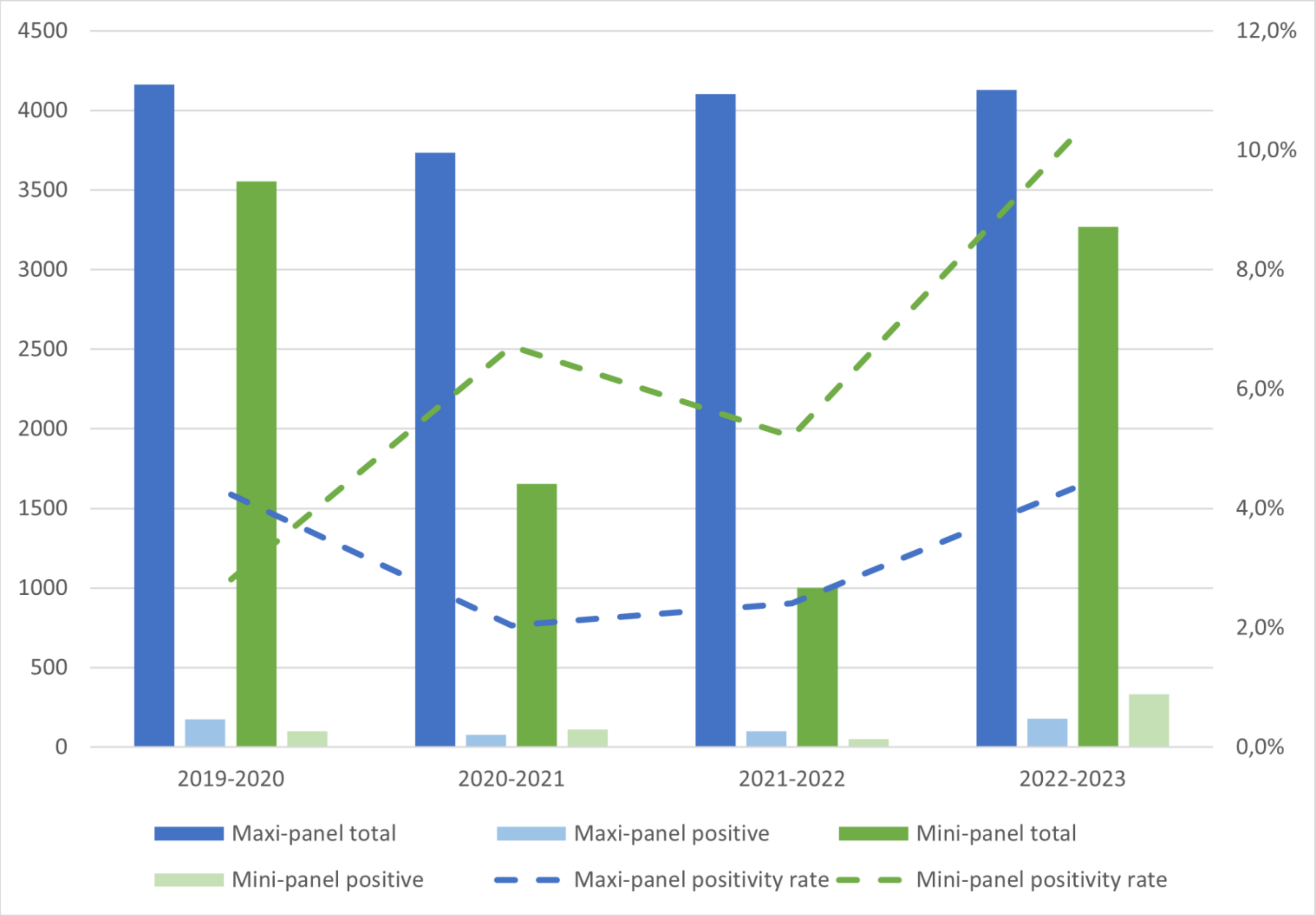
Positivity rate for RSV over time (UZ Leuven data)



Impact testing strategy:

- Maxi-panel: ~ **SARI** – accidential detection of RSV and lower PR – stable testing structure
- Mini-panel: ~ **ILI** – more targeted => implementation improves epidemiological surveillance of RSV – increasing positivity rate
  - Influenza/RSV PCR pre-COVID and early COVID (in combination with SARS-CoV-2 as separate assay)
  - Minipanel as of today: implemented in 2022 (more extended use)

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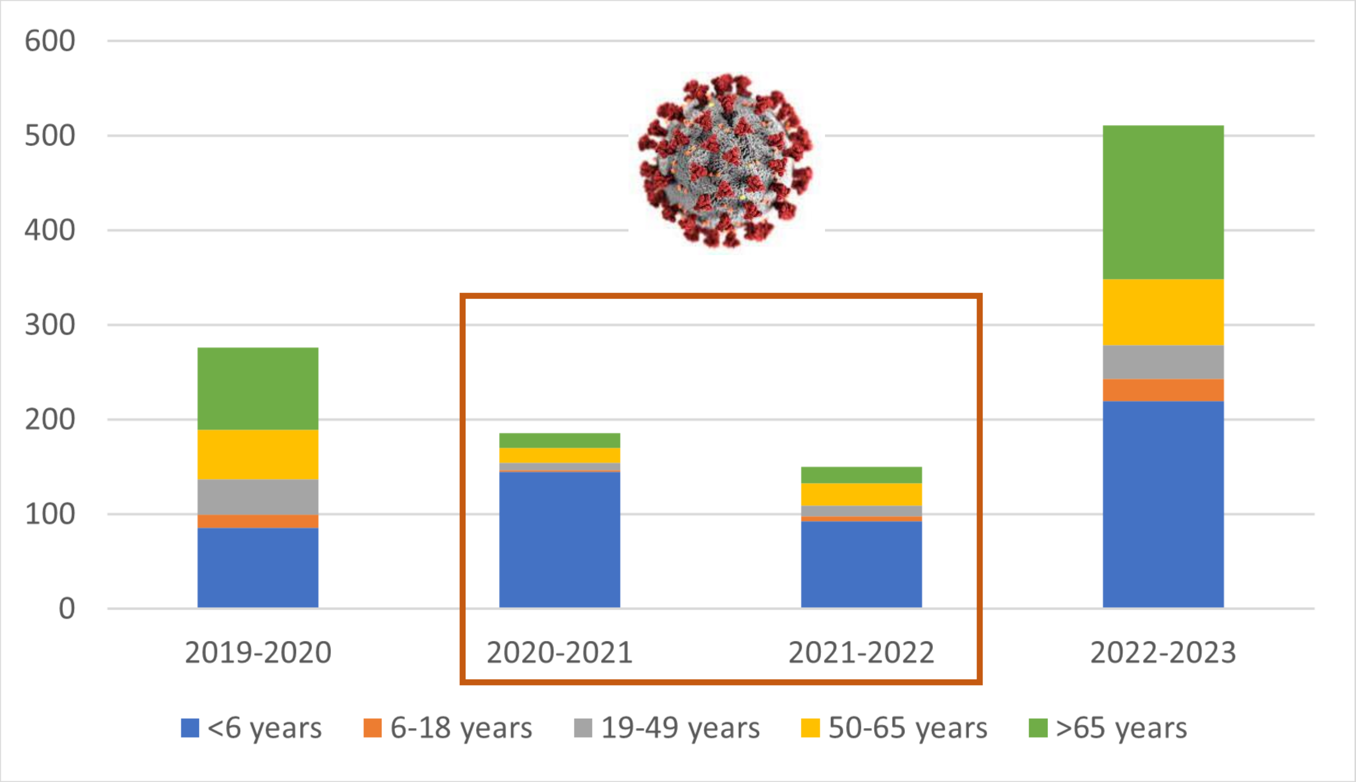
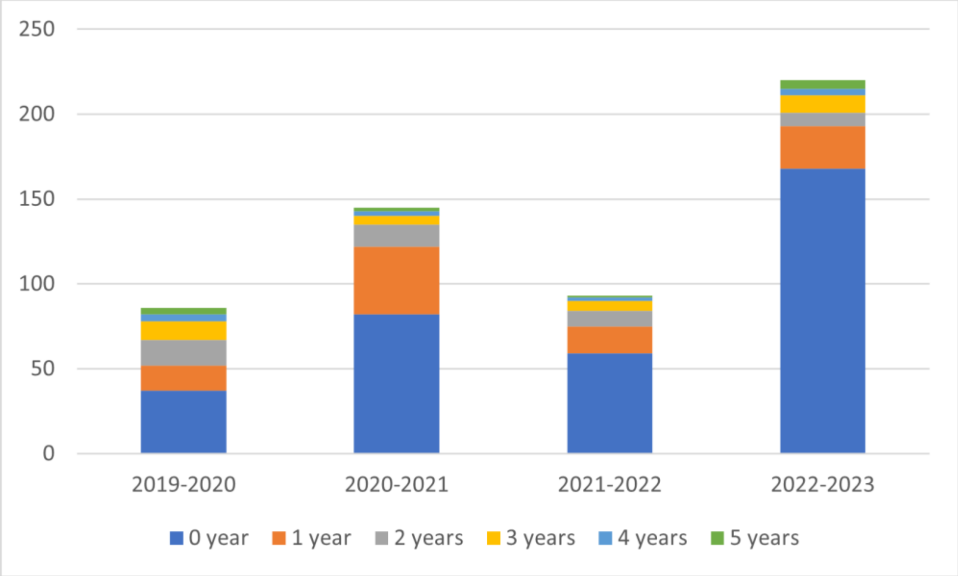
RSV detection mainly in infants (<2y) and elderly (>65y)

RSV positive cases detected by maxi- and minipanel





Impact testing strategy: PR and age distribution

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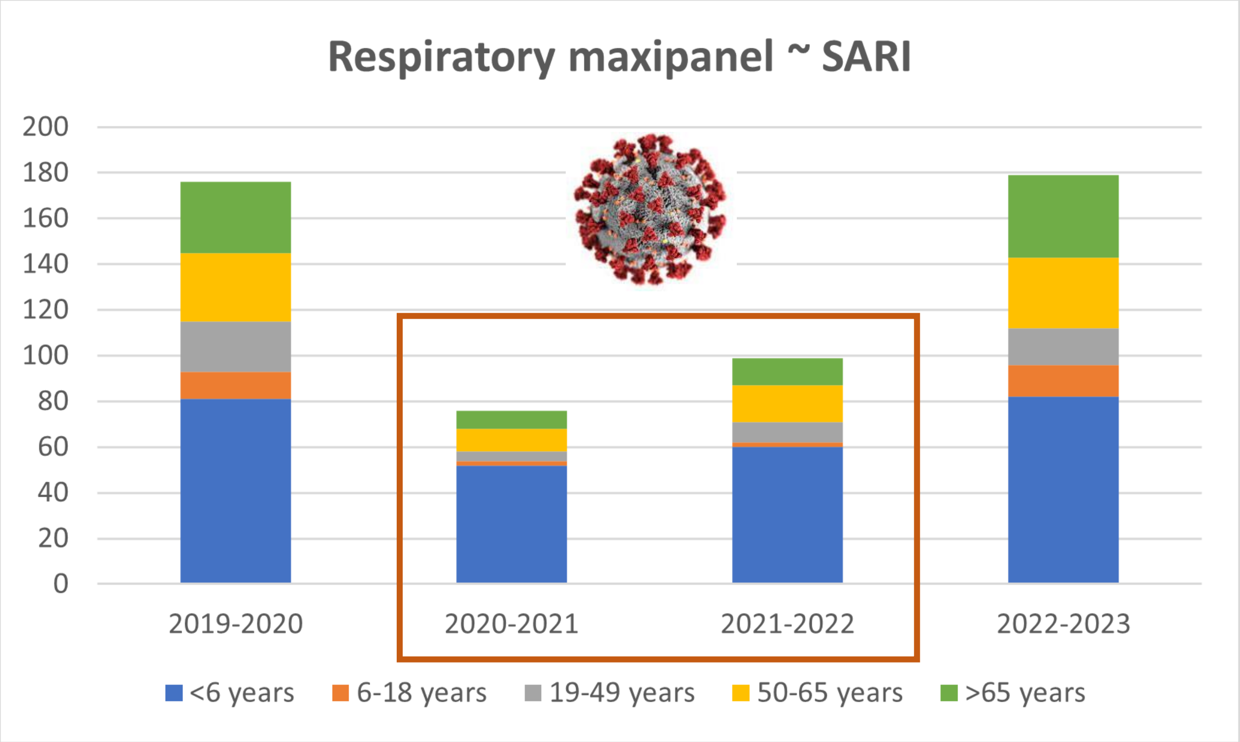
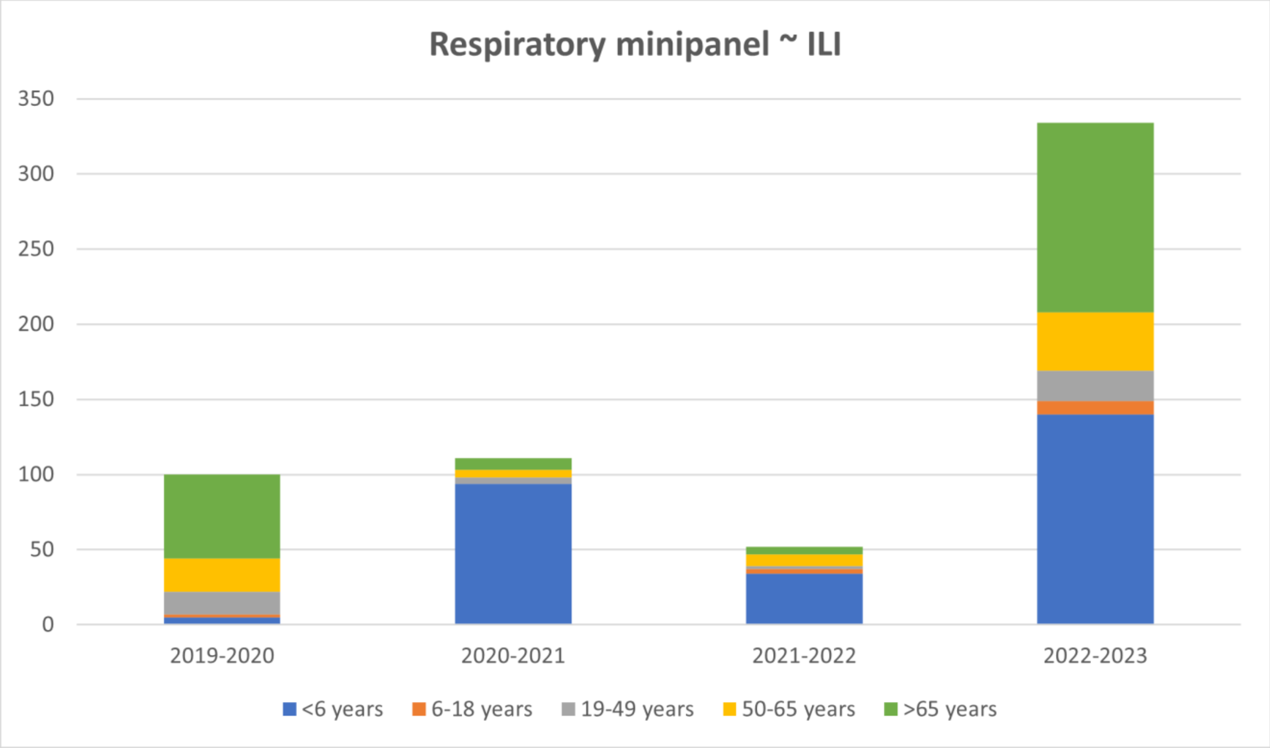
RSV positive cases according to detection method





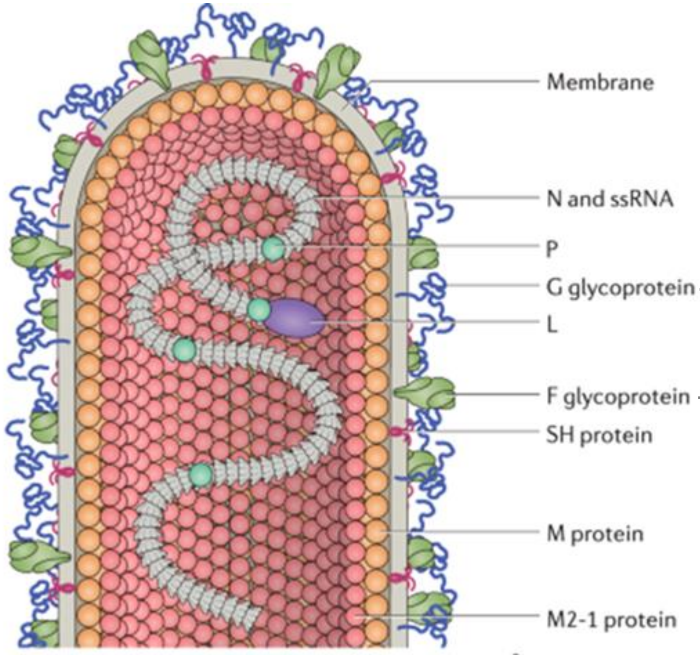
Impact COVID-19 pandemic: mild seasons 2020-2022

Switch influenza/RSV PCR (pre-COVID) to mini-panel (incl. SARS-CoV-2): impact on PR and age distribution
=> 2022-2023: risk populations RSV well represented

Molecular epidemiological analysis of HRSV

- Genomic surveillance of HRSV:
  - describe genetic evolution and geographical distribution of new variants
  - examine association of genotypes with disease severity
  - monitor evolution of viral proteins which are targets for diagnostic assays, mAb therapy, vaccines

- G (attachment protein) and F (fusion protein)
  - targets of host immune response
  - G is most variable: study genetic diversity
  - F is more conserved: target for vaccines & mAbs



G glycoprotein:
- variable across RSV subtypes & genotypes
- essential for infectivity in vivo
- induces neutralising Ab responses

F glycoprotein:
- required for cell entry
- highly conserved
- induces neutralising Ab responses
- targeted by most vaccine candidates and mABs

HRSV genome: ~15,2 kb ssRNA, 10 genes coding for 11 proteins

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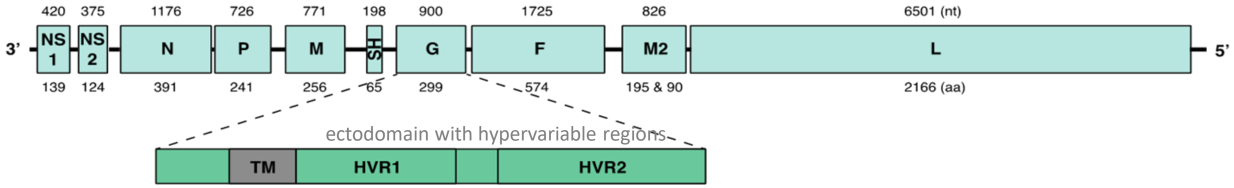
Molecular epidemiological analysis of HRSV

- Subtypes RSV-A and -B
  - diversity most extensive in G attachment glycoprotein (only 53% conserved on AA level)
  - co-circulation with variable subtype predominance in consecutive epidemic season
  - subtyping assay: RT-qPCR using subtype specific primer/probe sets



- Genotypes
  - multiple genotypes within each subtype
  - no consistent genotype definition/nomenclature, most classification systems based on ectodomain of G-gene or HVR2
  - proposals for new classification procedure, based on well-established phylogenetic methods:
    - Goya et al., 2020
    - Ramaekers et al., 2020

    → discussion on consensus ongoing
  - genotyping assay: RT-PCR + sequencing of G ectodomain, phylogenetic analysis

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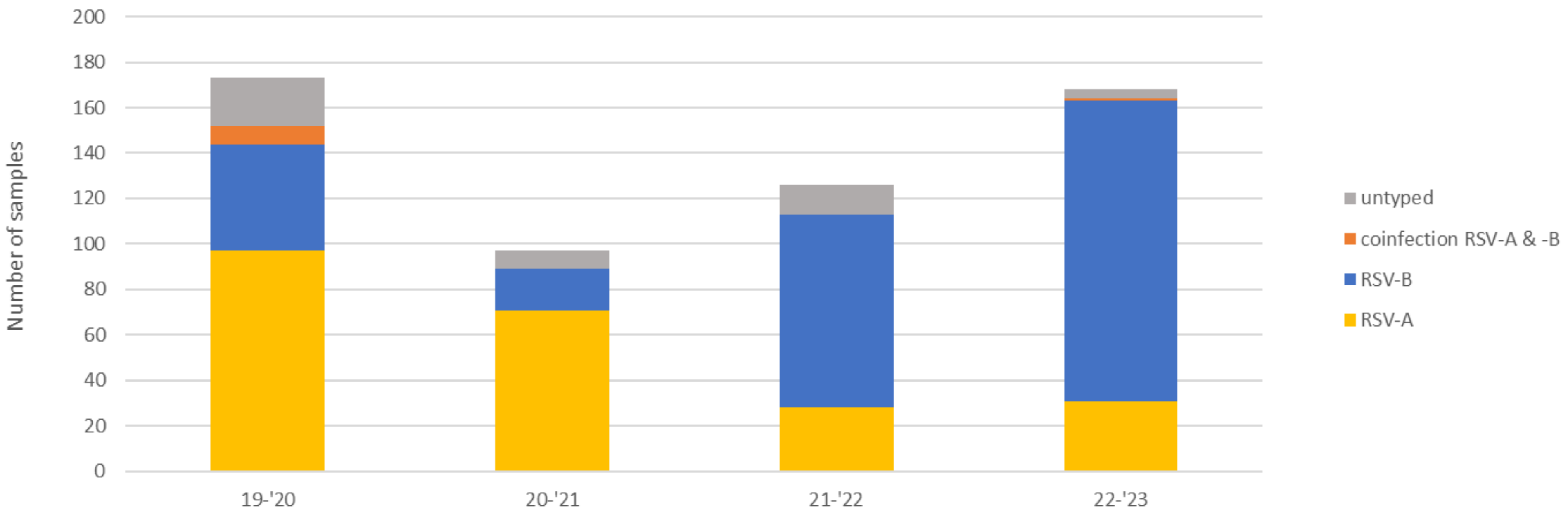
Molecular epidemiological analysis of HRSV: subtypes

- Subtype distribution season 2019-2020 until 2022-2023 (n=564):
  - co-circulation of RSV-A and -B during all 4 epidemic seasons
  - 2019-2020 & 2020-2021: predominance RSV-A
  - 2021-2020 & 2022-2023: predominance RSV-B



Molecular epidemiological analysis of HRSV: subtypes

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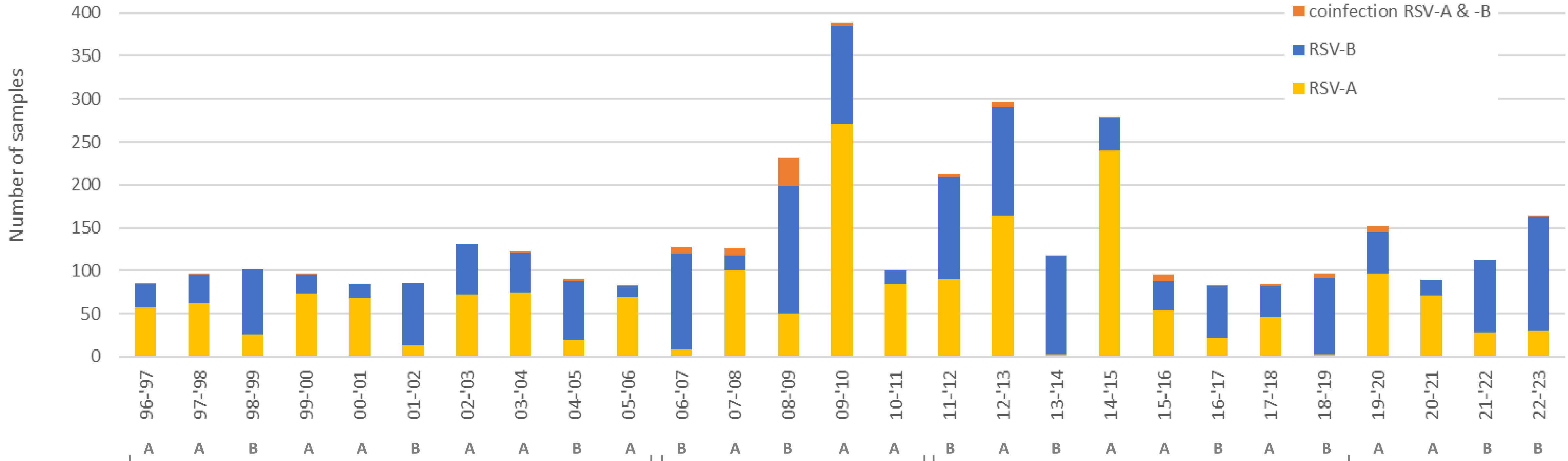
- Subtype distribution in Belgium since 1996



1996-2006:
3 yearly cyclic pattern (A-A-B)
Zlateva et al., 2007

2006-2010
yearly alternating (A-B-A-B)
Houspie et al., 2013

2011-2019
yearly alternating (A-B-A-B)
Ramaekers et al., in preparation

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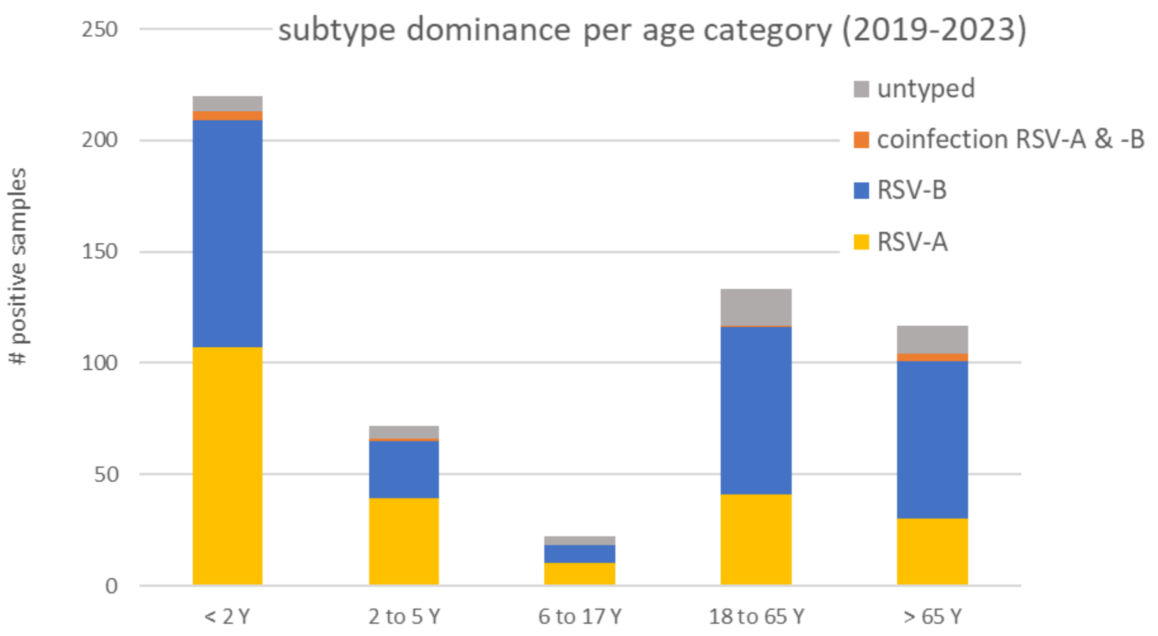
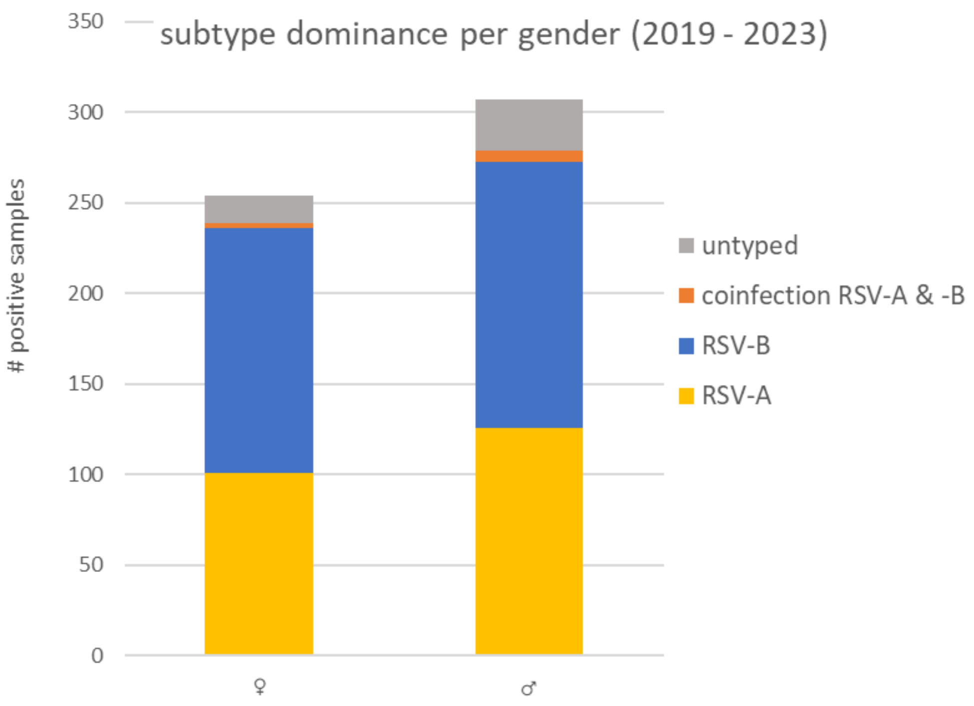
Molecular epidemiological analysis of HRSV: subtypes

- Subtype dominance per age category:
  - higher % RSV-A in younger age groups (<2Y, 2-5Y and 6-17Y)
  - was a trend in all 4 epidemic seasons

- Subtype dominance per gender:
  - no difference between ♂ and ♀





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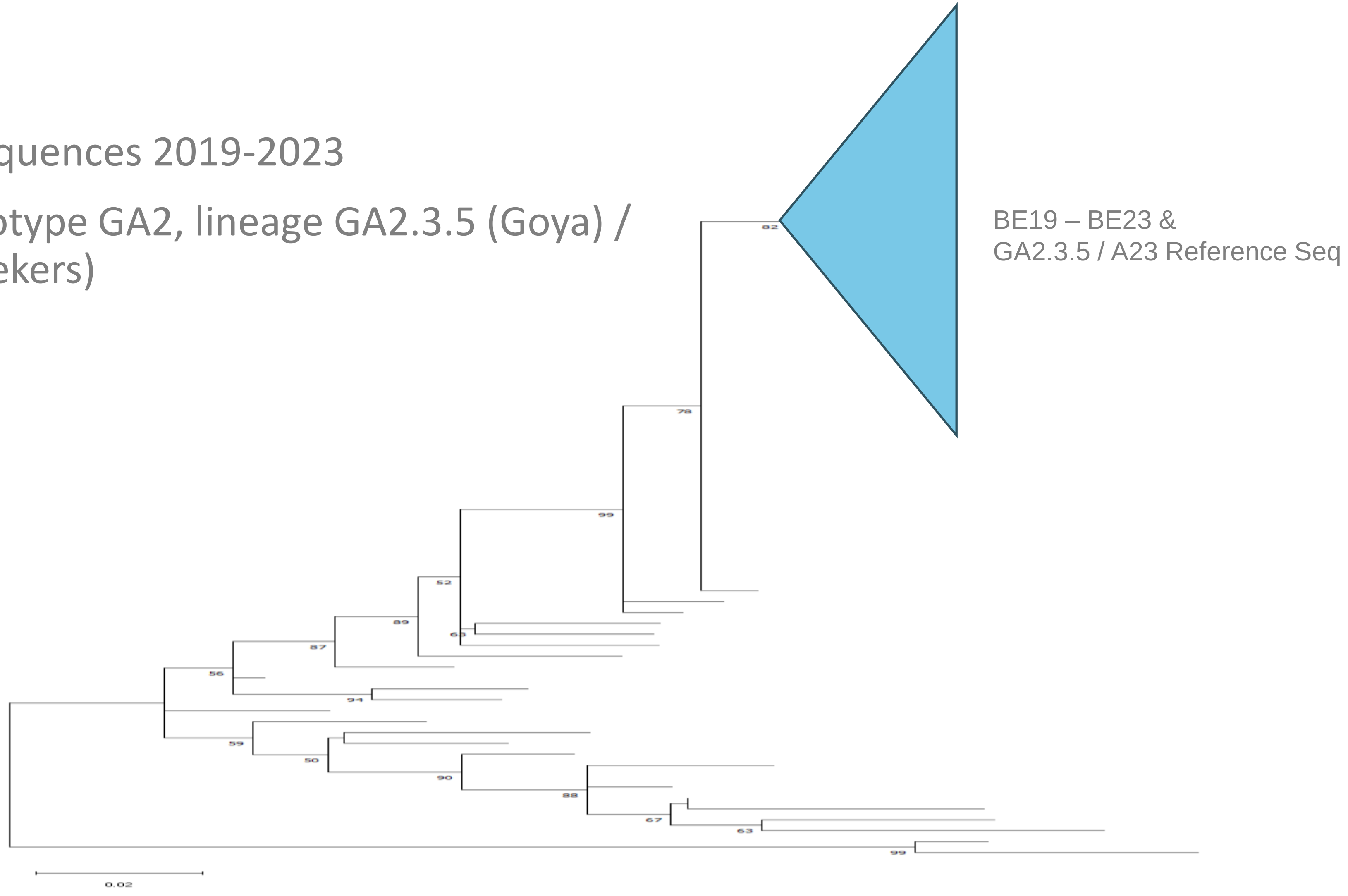
Molecular epidemiological analysis of HRSV: genotypes

RSV-A:

- 192 G ectodomain sequences 2019-2023
- all cluster within genotype GA2, lineage GA2.3.5 (Goya) / genotype A23 (Ramaekers)



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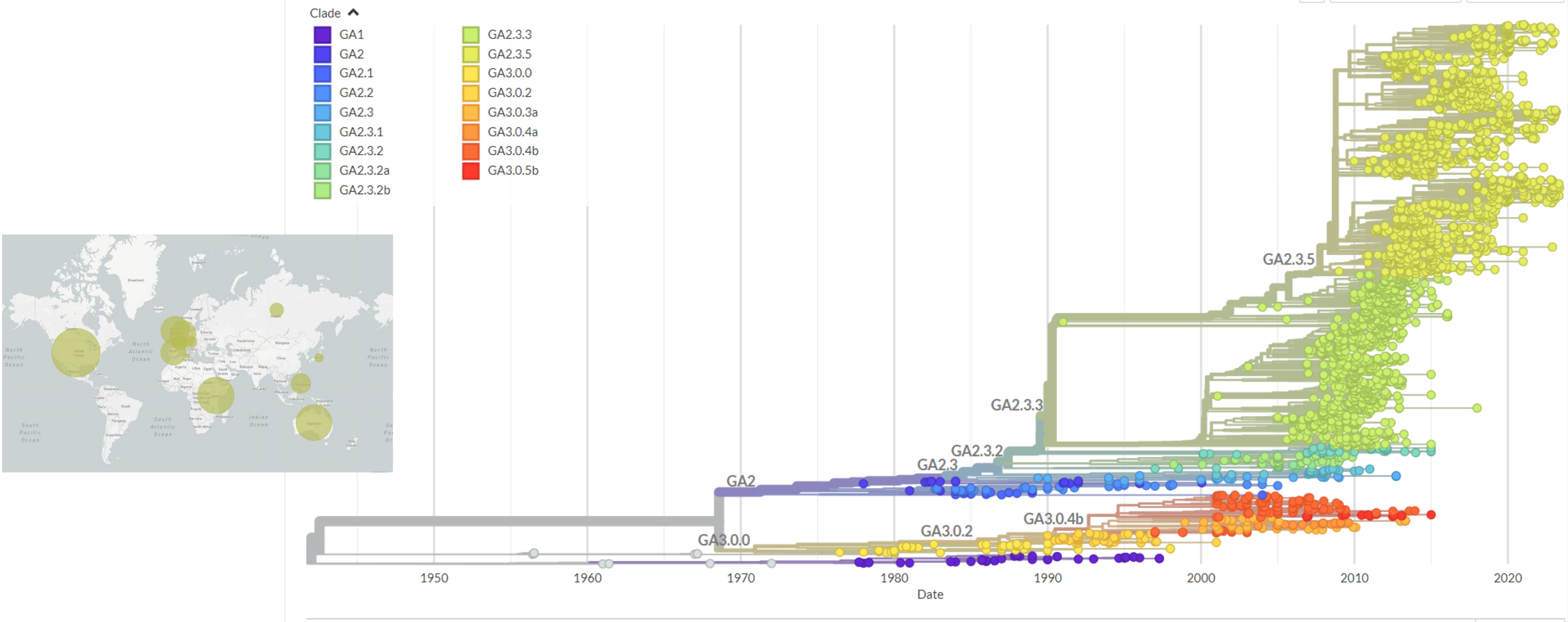
Molecular epidemiological analysis of HRSV: genotypes

Global perspective: RSV-A sequences 2019-2023 on Nextstrain → all lineage GA2.3.5 / genotype A23



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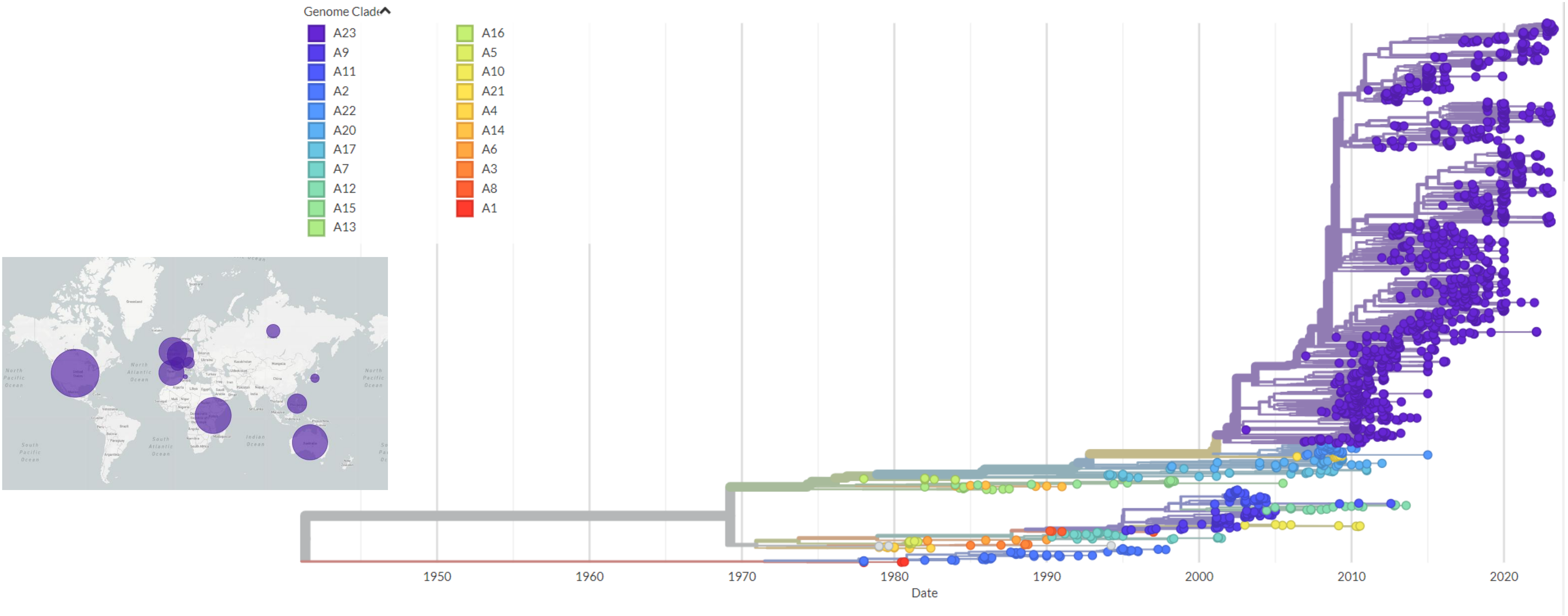
Molecular epidemiological analysis of HRSV: genotypes

Global perspective: RSV-A sequences 2019-2023 on Nextstrain → all lineage GA2.3.5 / genotype A23



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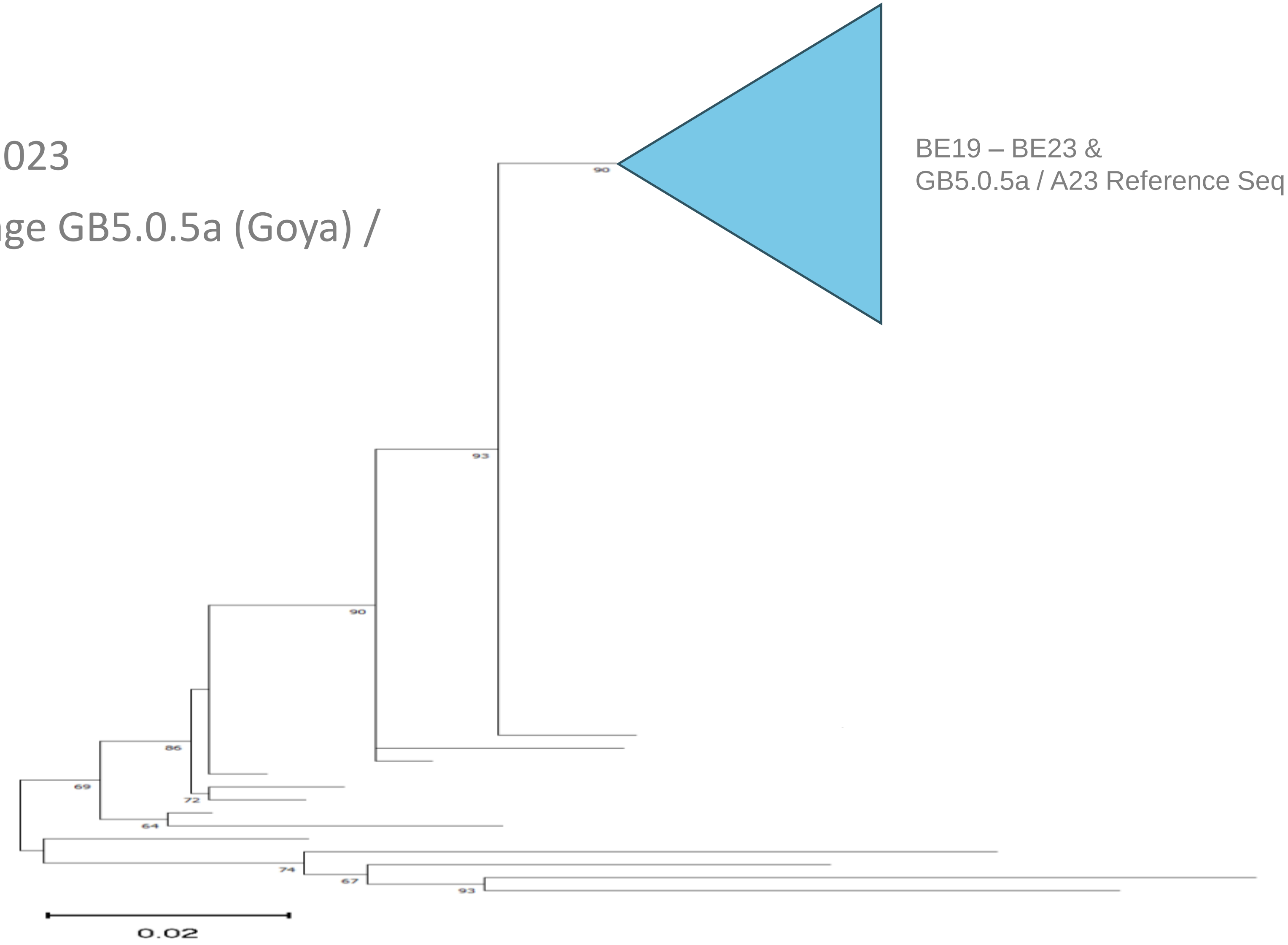
Molecular epidemiological analysis of HRSV: genotypes

RSV-B:

250 G ectodomain sequences 2019-2023

all cluster within genotype GB5, lineage GB5.0.5a (Goya) / genotype B6 (Ramaekers)



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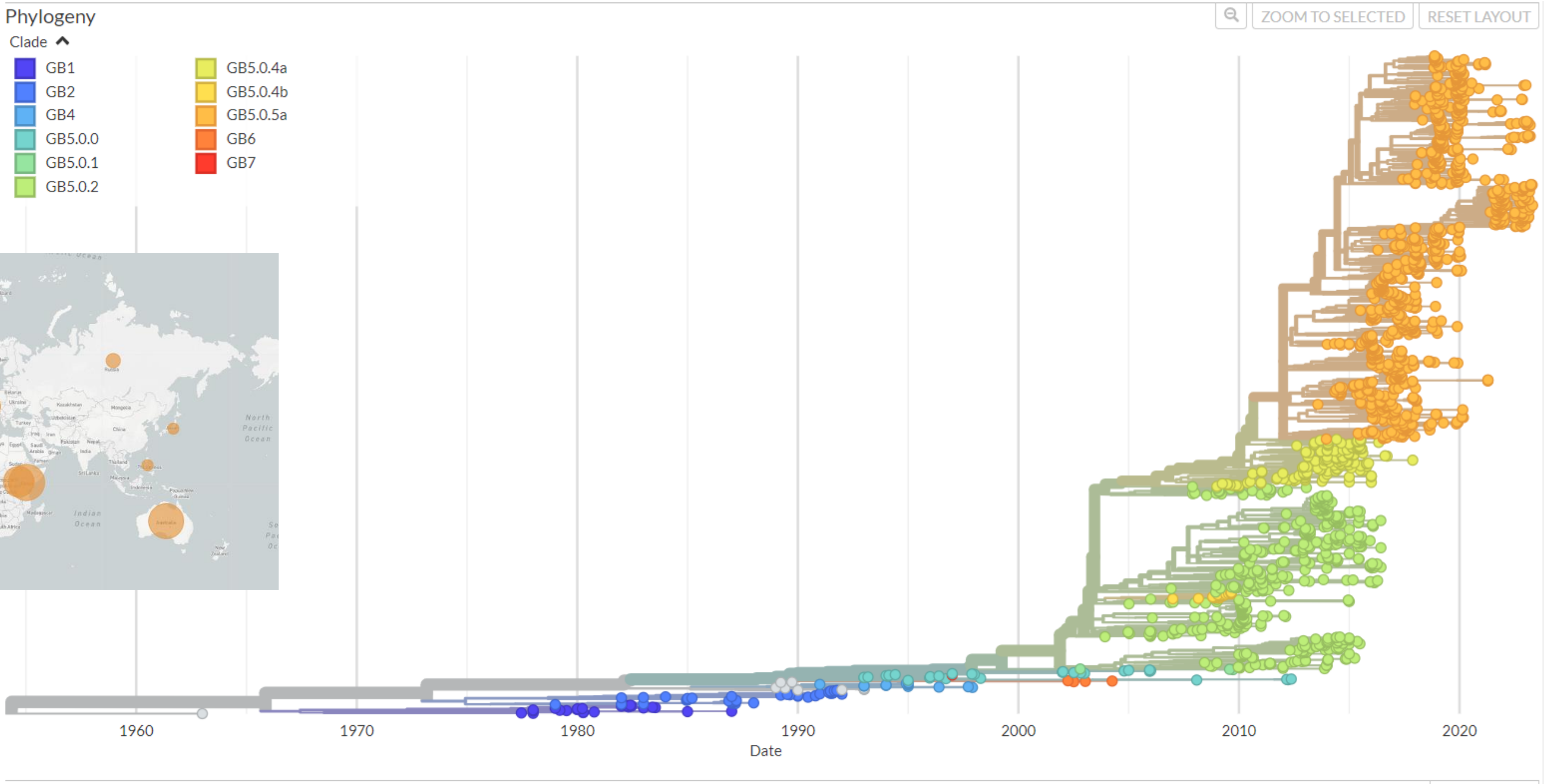
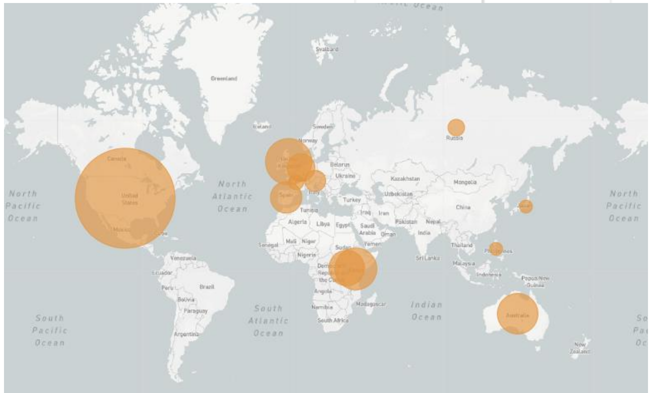
Molecular epidemiological analysis of HRSV: genotypes

Global perspective: RSV-B sequences 2019-2023 on Nextstrain → all lineage GB5.0.5a / genotype B6





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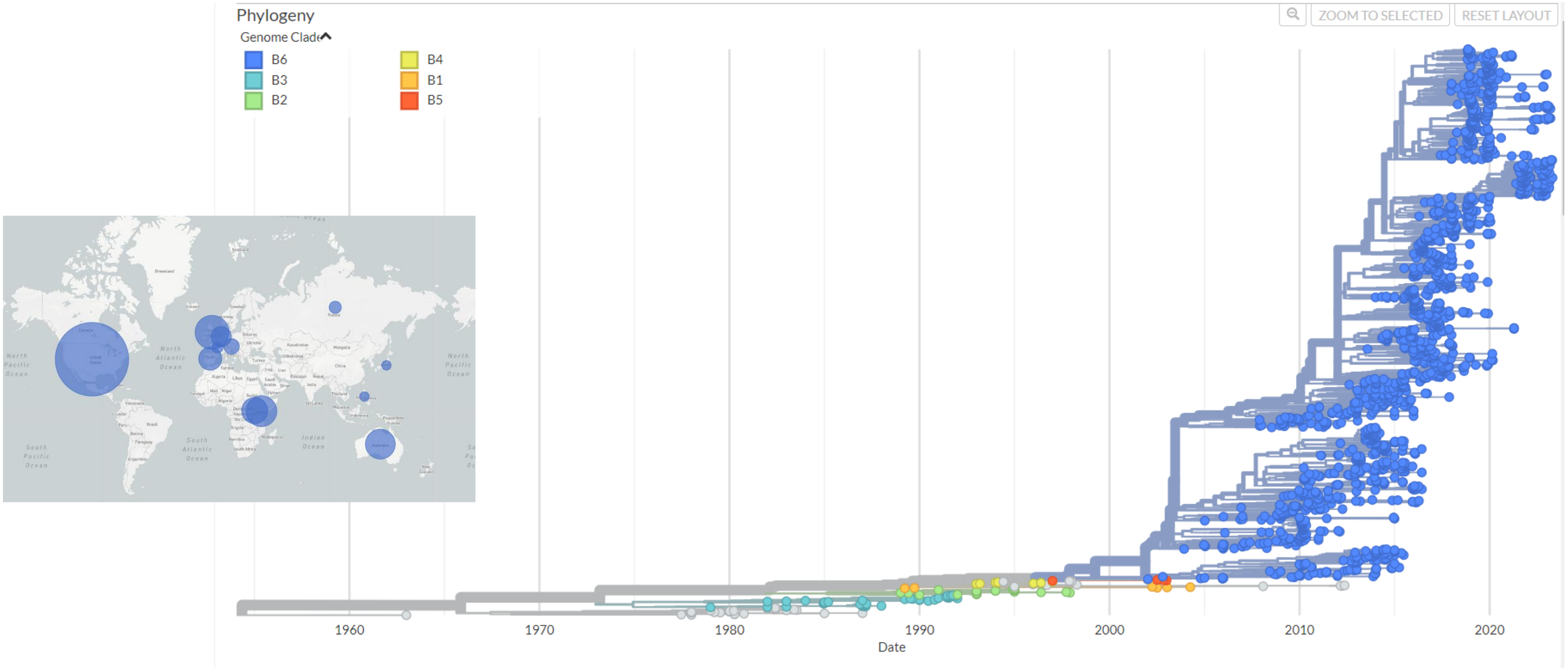
Respiratory pathogens

Molecular epidemiological analysis of HRSV: genotypes

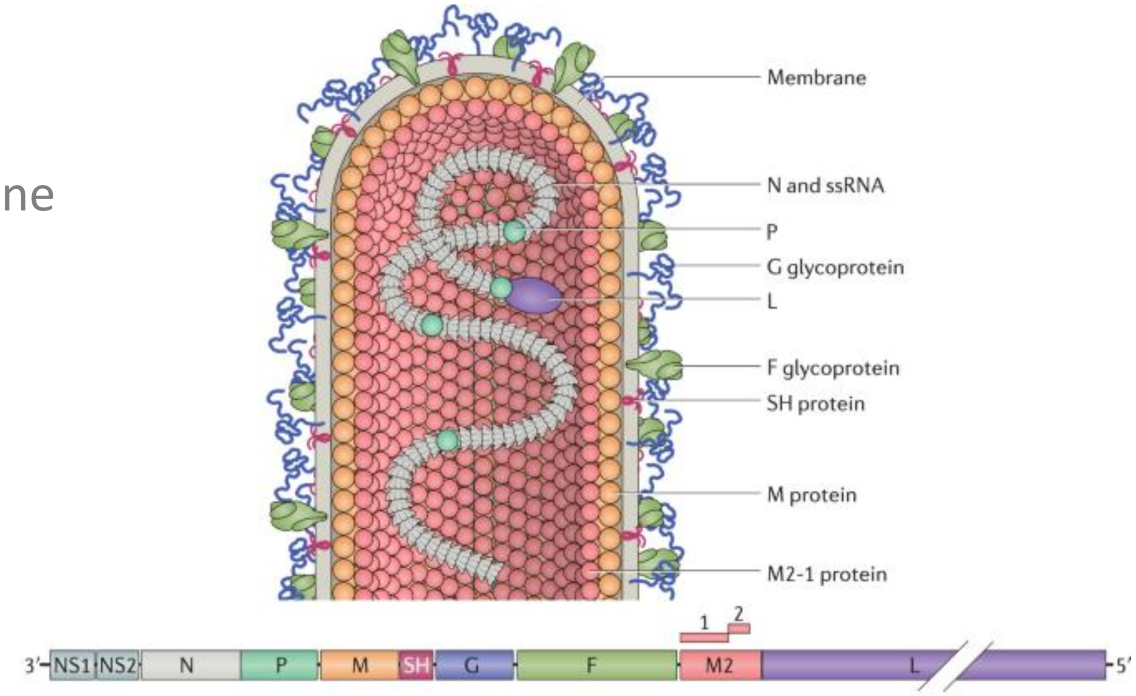
Global perspective: RSV-B sequences 2019-2023 on Nextstrain → all lineage GB5.0.5a / genotype B6



Molecular epidemiological analysis of HRSV: WGS

Need for more extensive sequencing data:

- RSV vaccines & monoclonal Abs target F protein: surveillance for vaccine/Ab escape mutations in F gene



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- Vaccines:

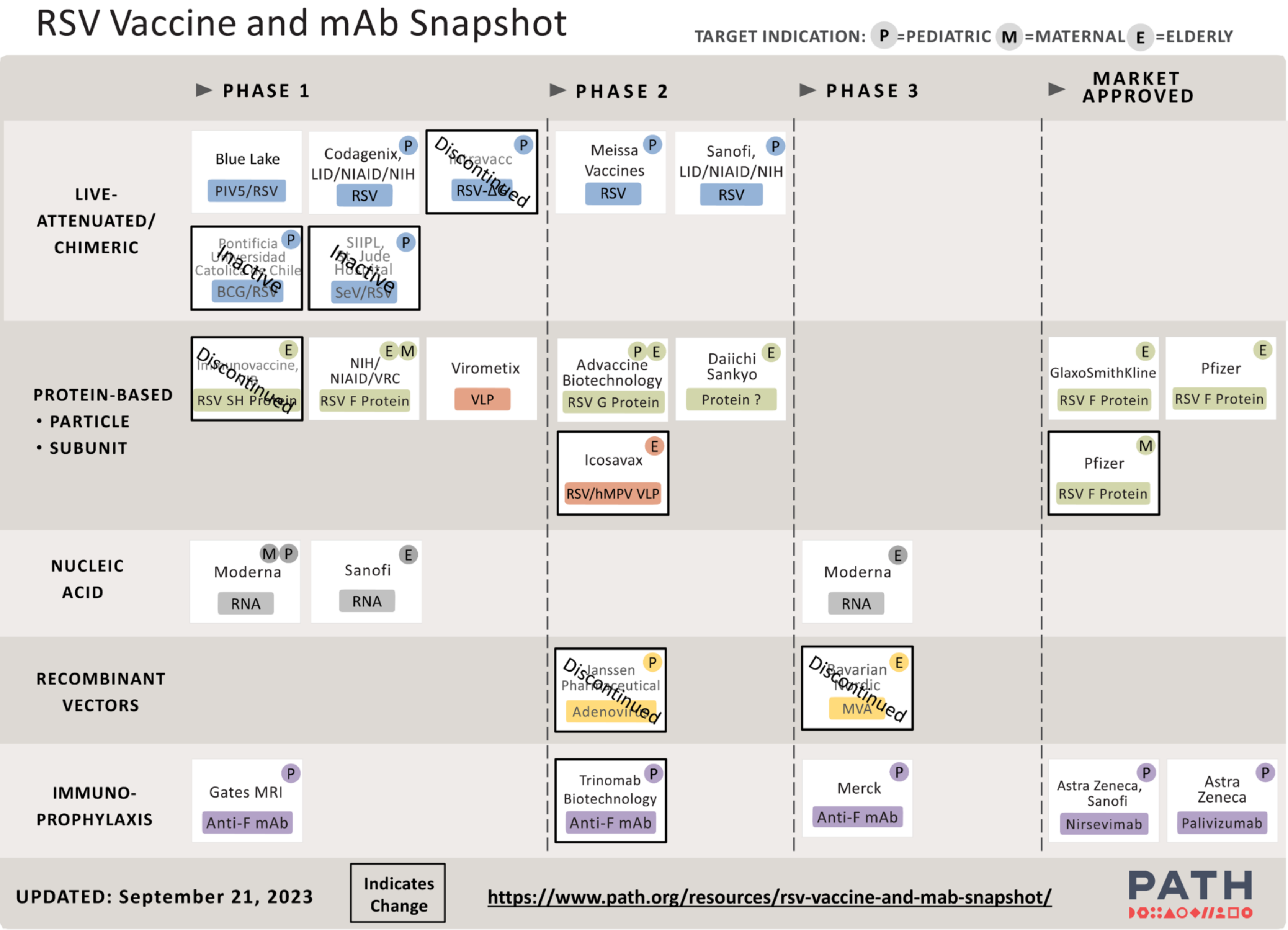
  Arexvy (GSK): elderly

  Abrysvo (Pfizer): elderly, pregnant women

- Immunoprophylactics:

  Palivizumab (Synagis®, AstraZeneca)

  Nirsevimab (Beyfortus®, AstraZeneca)



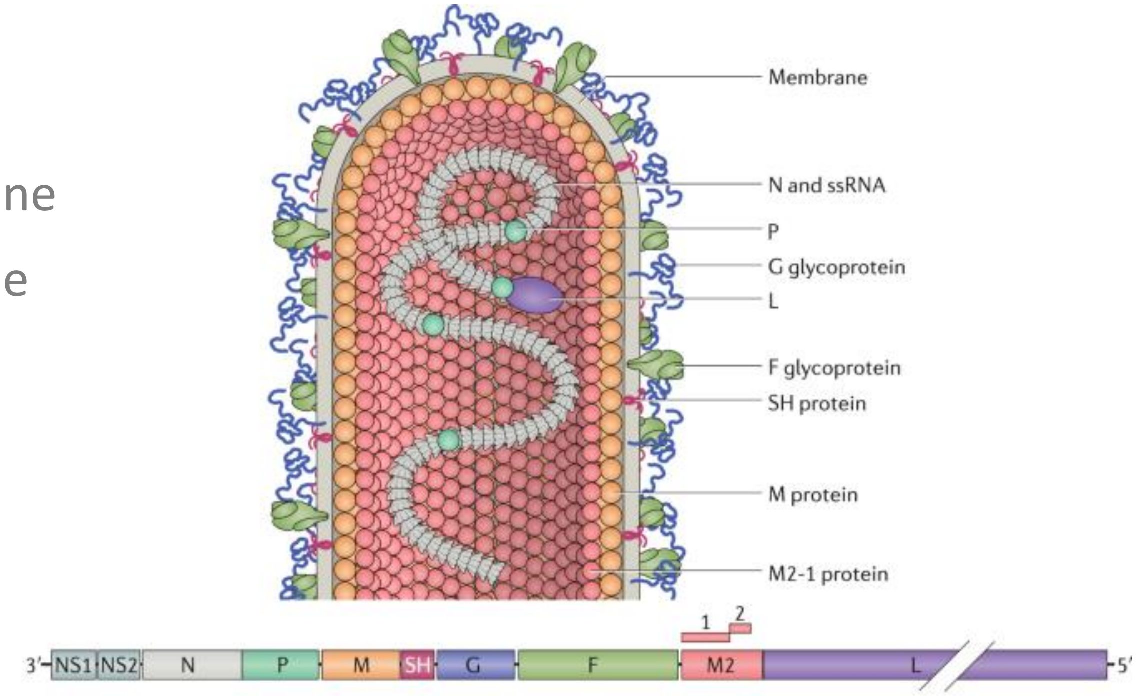
National Reference Center
Respiratory pathogens

Molecular epidemiological analysis of HRSV: WGS

Need for more extensive sequencing data:

- RSV vaccines & monoclonal Abs target F protein: surveillance for vaccine/Ab escape mutations in F gene
- Increasing knowledge on effects of mutations outside G and F on RSV evolution and Ab-based prevention strategies
- Reliable phylogeny: at least complete G-gene
  Clear genotype demarcation: complete genome

→ New consensus classification system:
  - phylogeny based on complete RSV genome
  - signature AA changes



➡ Whole genome sequencing for RSV: - currently being optimized in collaboration with ONT
- representative subset of circulating strains

Request for RSV positive samples for typing

- Request to send **RSV positive samples for molecular typing**
- Criteria:
  - Ct ≤ 20
  - Minimal volume of 700 microliters
  - Selection of 10 samples per season – ideally based on age:
    - <6 years age group: 5 samples
    - >65 years age group: 3 samples
    - Remaining 2 samples randomly from individuals aged 6-65 years
  - **Epidemiological surveillance <-> outbreak context**
- **NRC application form will be adapted** to also collect information on:
  - Details on PCR and obtained Ct value at the sending laboratory
  - Vaccination status of the patient
  - Use of monoclonal antibodies for infants
  - Addition of ‘RSV molecular typing’ as test: criteria and context

Detection and surveillance of respiratory pathogens in environmental samples: surveillance of wastewater

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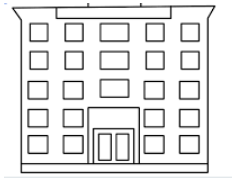
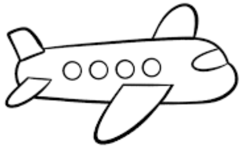
Respiratory viruses in environmental samples

- Broad detection of respiratory viruses in environmental samples as a complementary approach
  - strengthen the surveillance of respiratory pathogens
  - evaluate early warning signs
  - increase representativeness of the surveillance
- Air samples: collective sample at room/building level
- Wastewater samples:
  - respiratory viruses detectable in sewage water: eg. SARS-CoV-2, also other.
  - collective sample: 1 building/airplane/… → large geographical area’s





Respiratory viruses in environmental samples: sewage

- Leuven
- Kessel-Lo
- Heverlee
- Wilsele
- Wijgmaal
- Bertem
- Herent
- Linden

→ 8 different municipalities: +/- 120.000 inhabitants

Time-proportional automated sampler: 50 ml every 10 min → 500 ml 24-hour influent wastewater

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Respiratory viruses in environmental samples: sewage

Two year follow up: respiratory viruses in wastewater in comparison to clinical samples UZL

- wastewater:
  - PMMoV: control
  - respiratory panel: Ct values
- # positive clinical samples:
  - hospitalized patients
  - oro- and nasopharyngeal swabs, bronchial/endotracheal aspirates, bronchoalveolar lavages

⇨ Typical seasonal respiratory viruses:

fluctuations in wastewater ≈ positive samples in UZL and epidemiological data Sciensano

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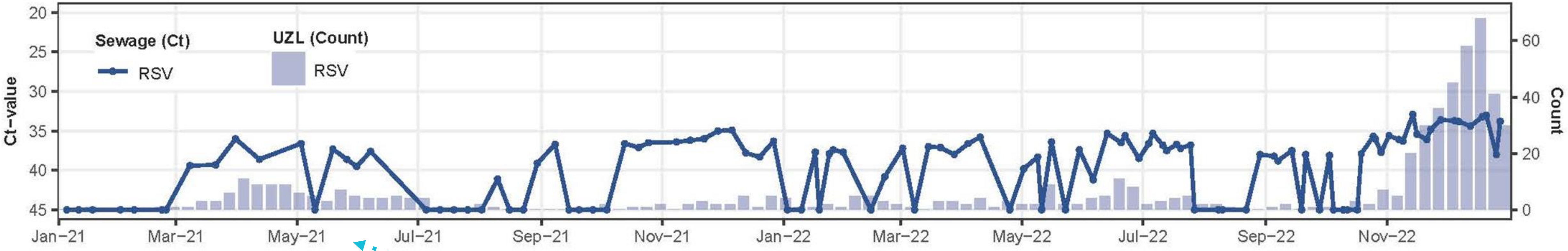
Respiratory viruses in environmental samples: sewage



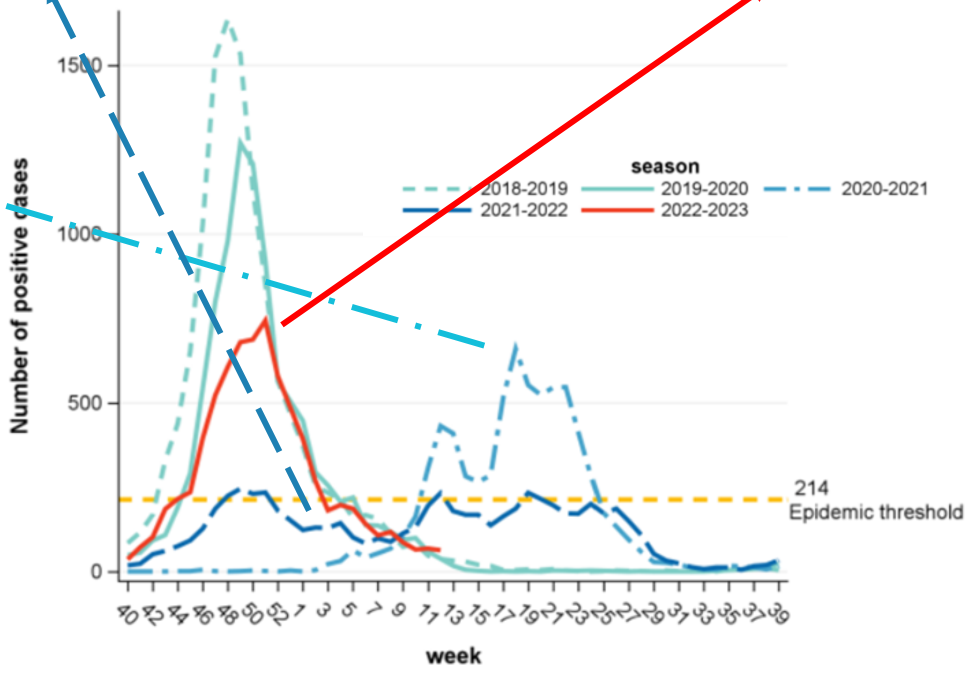
- RSV in wastewater:

  in accordance with # positive cases in UZL (bleu bars) and # positive test in sentinel labs (graph Sciensano)

  - RSV peak in spring 2021

  - low, continuous presence fall 2021 to July 2022

  - RSV peak in fall 2022



Respiratory viruses in environmental samples: sewage

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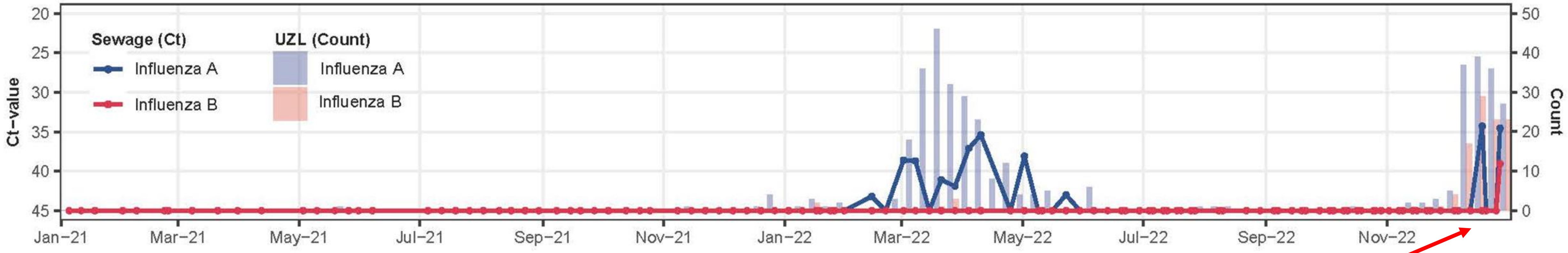


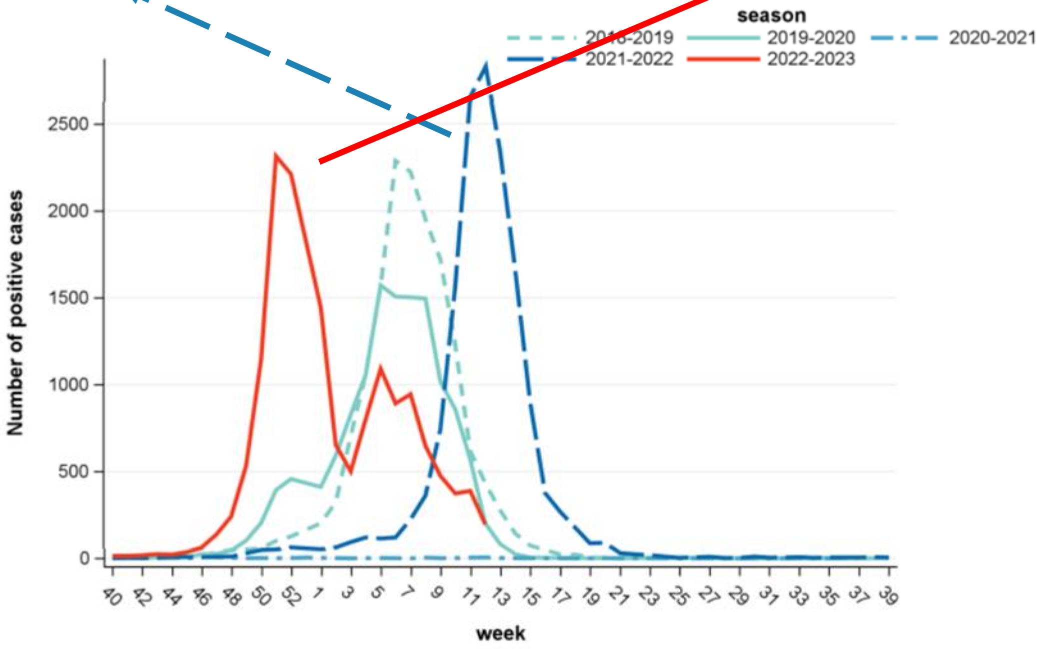
- influenza in wastewater:

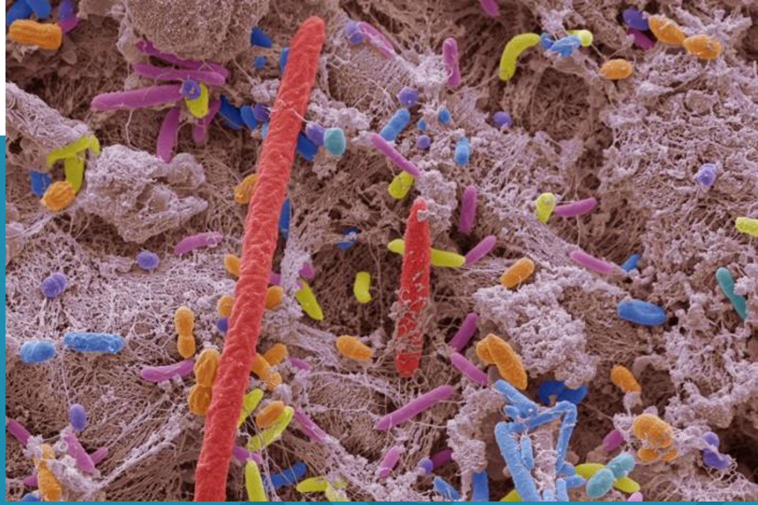
  in accordance with # positive cases in UZL (bleu/red bars) and # positive test in sentinel labs (graph Sciensano)

  - almost no influenza during winter 2020-2021

  - late influenza peak season 2021-2022 (spring 2022), 99% infl A

  - start influenza epidemic in Dec 2022, co-circulation infl A&B





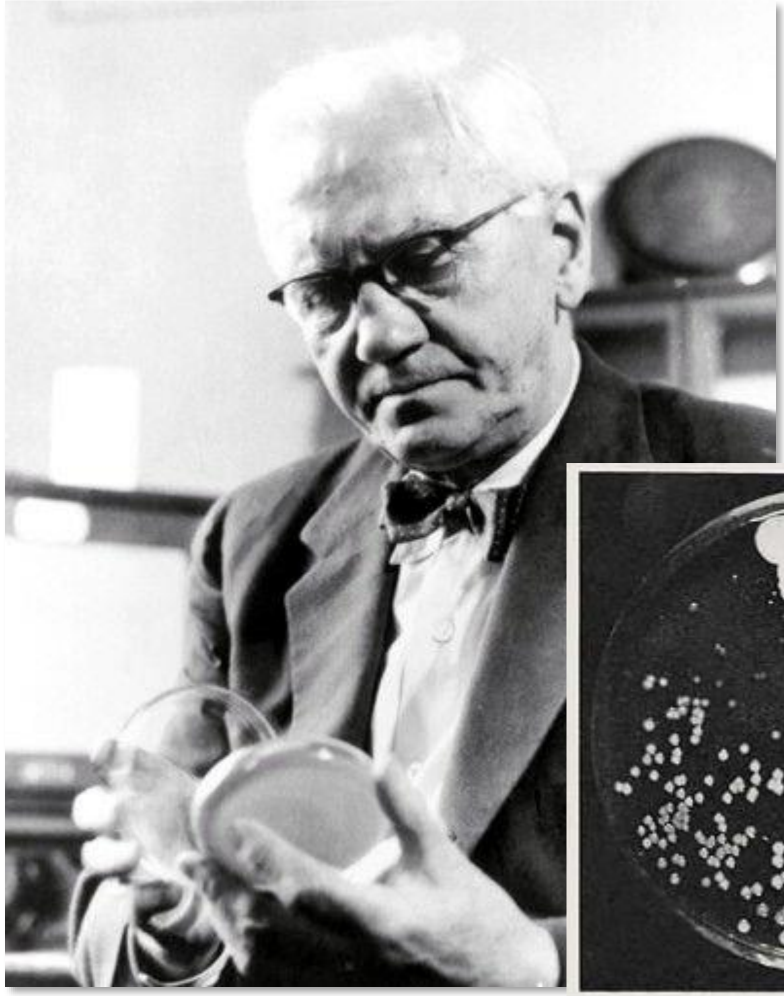
Indoor air microbiology, a precision tool for the surveillance of pathogens present in the air

An alternative for invasive and costly individual sampling?

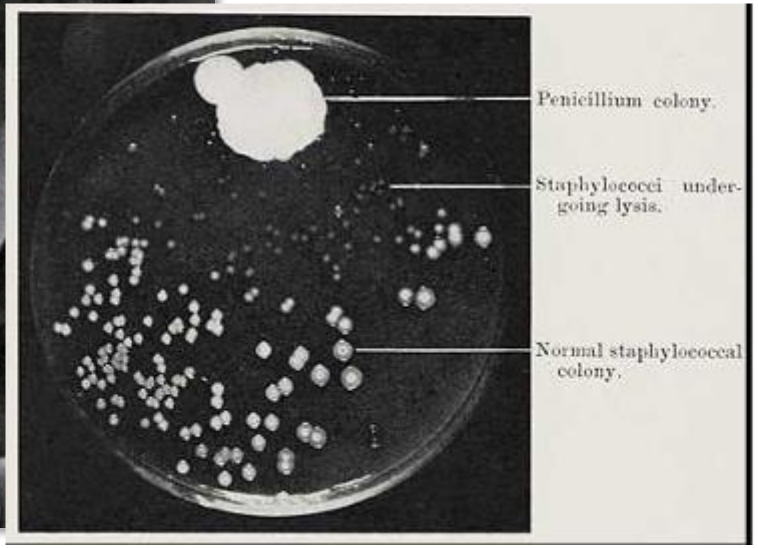
Laboratory of Clinical Microbiology

Indoor air microbiology: nothing new here!

Authentic 16th century plague doctor mask preserved and on display at the Deutschen Medizinhistorischen (German Museum of Medical History) in Ingolstadt



1928



Can we measure the risk of infection in an indoor setting?
Can we measure the impact of risk-reduction strategies?

nature communications

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Article | Open Access | Published: 11 March 2023

Indoor air surveillance and factors associated with respiratory pathogen detection in community settings in Belgium

Joren Raymenants, Caspar Geenen, Lore Budts, Jonathan Thibaut, Marijn Thijssen, Hannelore De Mulder, Sarah Gorissen, Bastiaan Craessaerts, Lies Laenen, Kurt Beuselinck, Sien Ombelet, Els Keyaerts & Emmanuel André

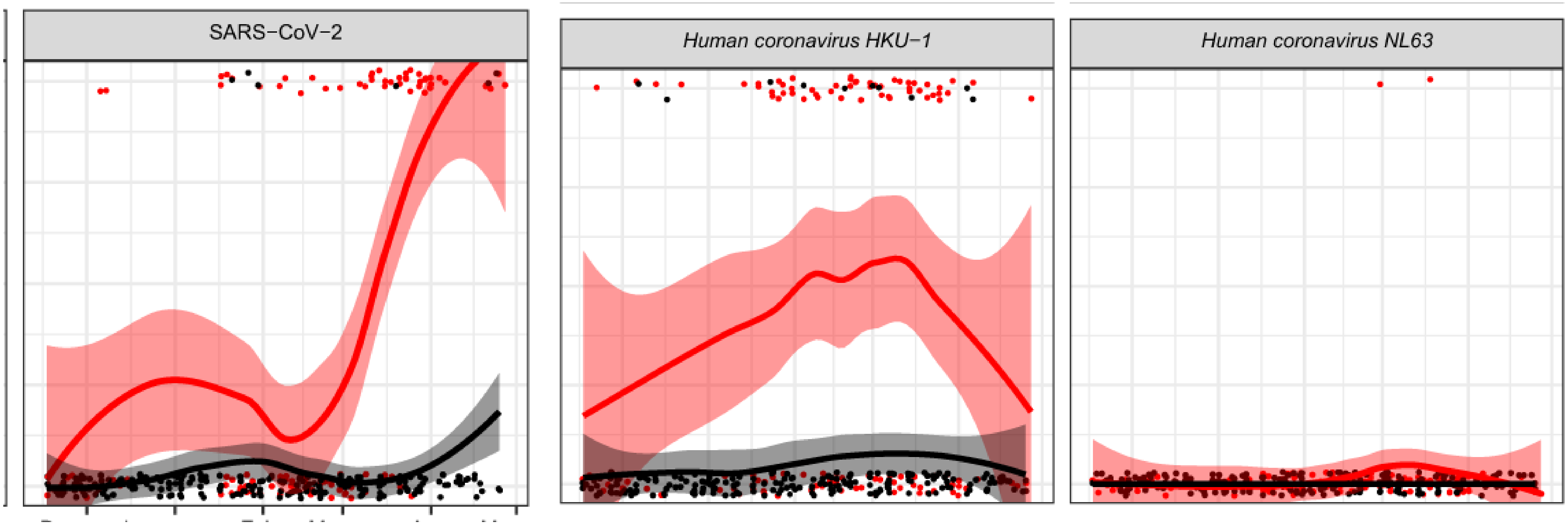
Nature Communications **14**, Article number: 1332 (2023) | Cite this article

Pathogen concentration and air quality

	p-value	Coefficient and 95% CI
CO_2	<0.0001	−0.08 (CI −0.12 to −0.04) per increase of 100 ppm
Portable air filtration	0.0005	0.58 (CI 0.25–0.91)

qPCR Ct of positive samples, 29 pathogens in a linear regression model

Positivity rates from indoor air of a nursery (red) and a nearby hospital (grey)



Can indoor air surveillance be incorporated in the architectural design of public spaces?

Can we monitor the epidemiology and anticipate outbreaks in targetted (vulnerable) populations?

Can indoor air surveillance be incorporated in the architectural design of public spaces?

Preprints with THE LANCET

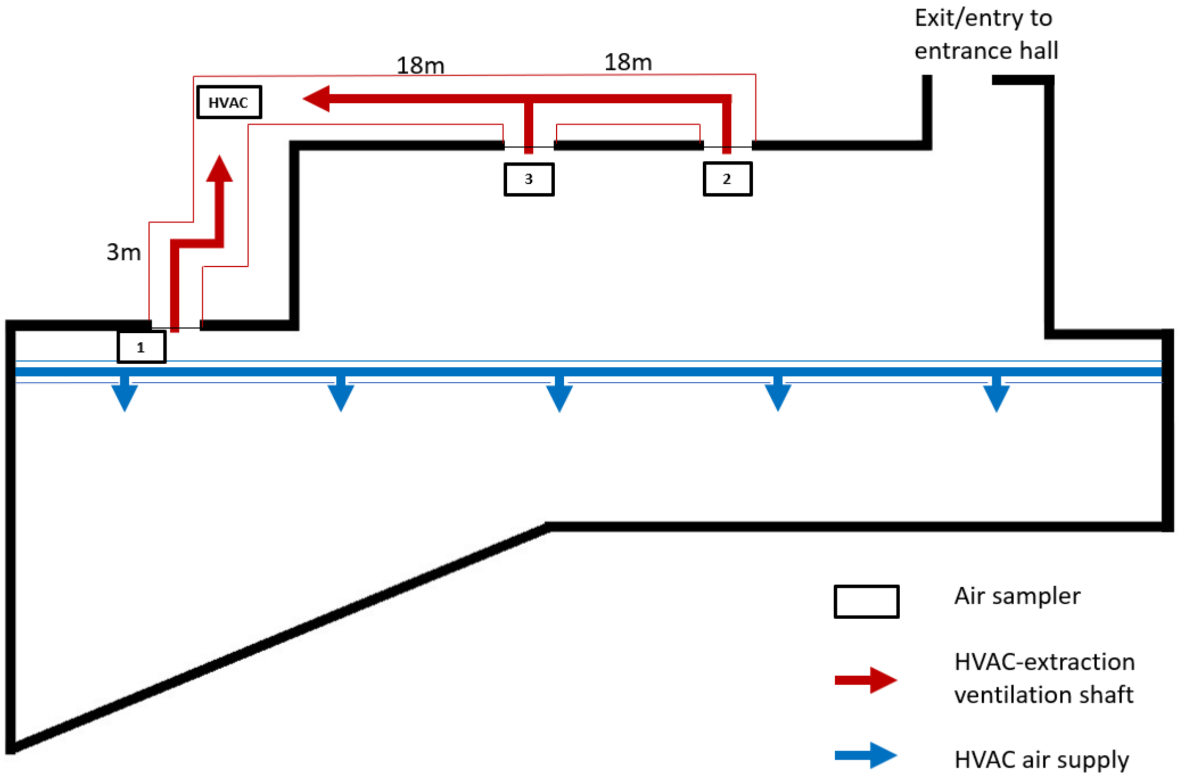
Preprints with The Lancet is part of SSRN´s First Look, a place where journals identify content of interest prior to publication. Authors have opted in at submission to The Lancet family of journals to post their preprints on Preprints with The Lancet. The usual SSRN checks and a Lancet-specific check for appropriateness and transparency have been applied. Preprints available here are not Lancet publications or necessarily under review with a Lancet journal. These preprints are early stage research papers that have not been peer-reviewed. The findings should not be used for clinical or public health decision making and should not be presented to a lay audience without highlighting that they are preliminary and have not been peer-reviewed. For more information on this collaboration, see the comments published in The Lancet about the trial period, and our decision to make this a permanent offering, or visit The Lancet´s FAQ page, and for any feedback please contact preprints@lancet.com.

Centralised Air Sampling from a Ventilation System for the Surveillance of Respiratory Pathogens

17 Pages • Posted: 10 Oct 2023

Michiel Happaerts
KU Leuven

Caspar Geenen
KU Leuven



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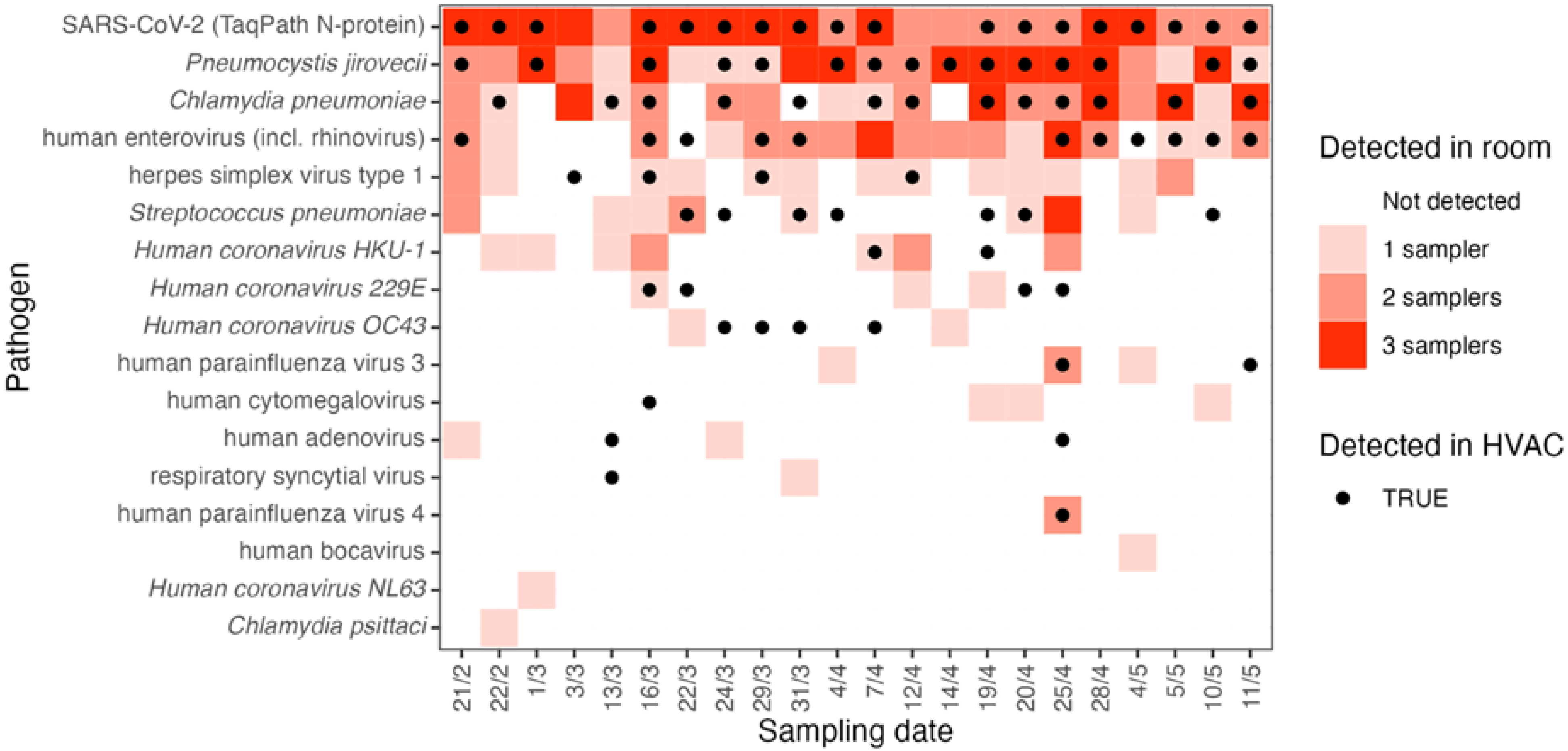
Results



SARS-CoV-2 (TaqPath N-protein)
Pneumocystis jirovecii
Chlamydia pneumoniae
human enterovirus (incl. rhinovirus)
herpes simplex virus type 1
Streptococcus pneumoniae
Human coronavirus HKU-1
Human coronavirus 229E
Human coronavirus OC43
human parainfluenza virus 3
human cytomegalovirus
human adenovirus
respiratory syncytial virus
human parainfluenza virus 4
human bocavirus
Human coronavirus NL63
Chlamydia psittaci
Pathogen
21/2 22/2 1/3 3/3 13/3 16/3 22/3 24/3 29/3 31/3 4/4 7/4 12/4 14/4 19/4 20/4 25/4 28/4 4/5 5/5 10/5 11/5
Sampling date
Detected in room
Not detected
1 sampler
2 samplers
3 samplers
Detected in HVAC
TRUE

Can we monitor the epidemiology and anticipate outbreaks in targetted (vulnerable) populations?

Air sampling

+

Used paper tissues

Correlation?

KU LEUVEN

Air sampling and individual samples

- Childcare setting
  - Regular attendees (children 0-3 years)
  - Weekly air sampling
  - Paper tissue sampling: 21 participants
  - PCR: respiratory panel