

Environmental detection of *Pneumocystis jirovecii* in different indoor settings highlights the potential role of children under 3 years and young adults in transmission

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Background

Pneumocystis jirovecii is an opportunistic pathogen. Most humans develop antibodies against *Pneumocystis jirovecii* at an early age.

It remains uncertain whether *Pneumocystis jirovecii* tends to colonize immuno-competent hosts long-term or if re-infections occur.

Research is hampered by:

- subclinical presentations of *Pneumocystis jirovecii* secondary infections
- invasive sampling procedures required for diagnosis
- inability of serological tests to capture re-infections

We here explore environmental air sampling to gather evidence of *Pneumocystis jirovecii* shedding by asymptomatic carriers as a way to assess exposure.

Methods

Congregate settings:

- Nursery (0-3y)
- Kindergarten (3-6y)
- Primary school (6-12y)
- Secondary school (12-18y)
- University (18+)
- Nursing homes (65+)



AerosolSense Sampler
(Thermo Fisher Scientific)



Sample cartridge
(Thermo Fisher Scientific)



UTM
(Copan)

LDT respiratory panel multiplex
qPCR,
Including *Pneumocystis jirovecii*

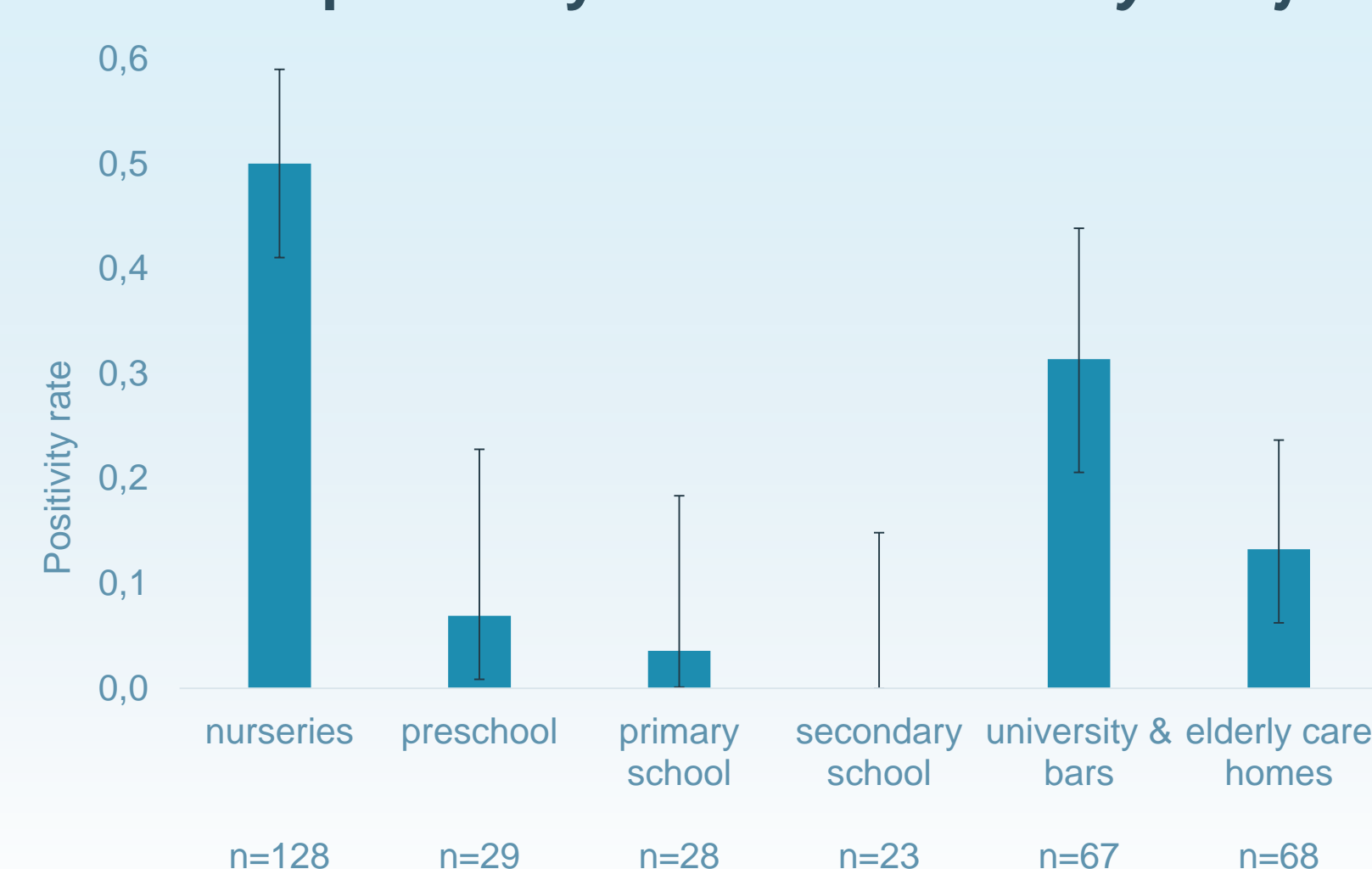
Primer/probe	sequence (5'→3')	Target gene	amplicon size
Forward primer	GCACTGAATATCTCGAGGGAGTATG	Large subunit ribosomal RNA gene	145 bp
Reverse primer	TTGGGAGCTTTAATTACTGTTCTCG		
Probe	FAM-TGTTTCCTTCCTTCGACTATC-MGB		

Results

SamplingSite	Predominant age	Room volume (m3)	HVAC present	Aimed sampling duration (h)	Sampling period	Total n samples
Nursery location 1	0y-3y	150	NO	2	15/11/21-18/01/23	106
Nursery location 2	0y-3y	157,5	NO	2	17/01/22-01/04/22	14
Nursery location 3	0y-3y	157,5	NO	2	07/02/22-01/04/22	8
Preschool cafeteria	3y-6y	372	NO	2	22/11/21-04/02/22	26
Preschool classroom	3y-6y	210	NO	2	08/12/21-02/02/22	3
Primary school cafeteria	6y-12y	930	NO	1-2	22/11/21-01/02/22	25
Primary school classroom	6y-12y	224	NO	1	08/12/21-02/02/22	3
Secondary school cafeteria	12y-18y	912	NO	2	23/11/21-28/03/22	23
University bar 1	18y-25y	200	NO	2	14/10/21-27/10/21	7
University bar 2	18y-25y	315	NO	2	18/10/21-10/11/21	7
University bar 3	18y-25y	198	YES	2	18/10/21-10/11/21	10
University cafeteria	18y-25y	7614	YES	2	18/10/21-29/10/21	10
University auditorium 1	18y-25y	324	NO	2	01/12/21-08/12/21	3
University auditorium 2	18y-25y	324	NO	2	04/11/21-25/11/21	13
University auditorium 3	18y-25y	324	NO	2	08/11/21-13/12/21	6
University auditorium 4	18y-25y	731,25	NO	2	14/12/21-14/12/21	1
Sports club bar	18y-65y	324	NO	2	20/01/22-31/03/22	10
Elderly care home 1	+65y	273	YES	2	09/12/21-01/02/22	19
Elderly care home 2	+65y	627,75	YES	2	06/12/21-04/02/22	35
Elderly care home 3a	+65y	600	YES	2	06/01/22-31/01/22	8
Elderly care home 3b	+65y	600	YES	2	06/01/22-24/01/22	6

Overview of sampling sites characteristics with predominant age, room volume, presence of HVAC (heating, ventilation and air conditioning), aimed sampling duration, sampling period, and number of samples successfully analyzed. Of the total 391 indoor air samples collected, 41 samples were excluded because of use of an active air purifier. Another 7 samples were excluded due to logistics mistakes and technical errors. Table adapted from Raymenants et al. 2023.

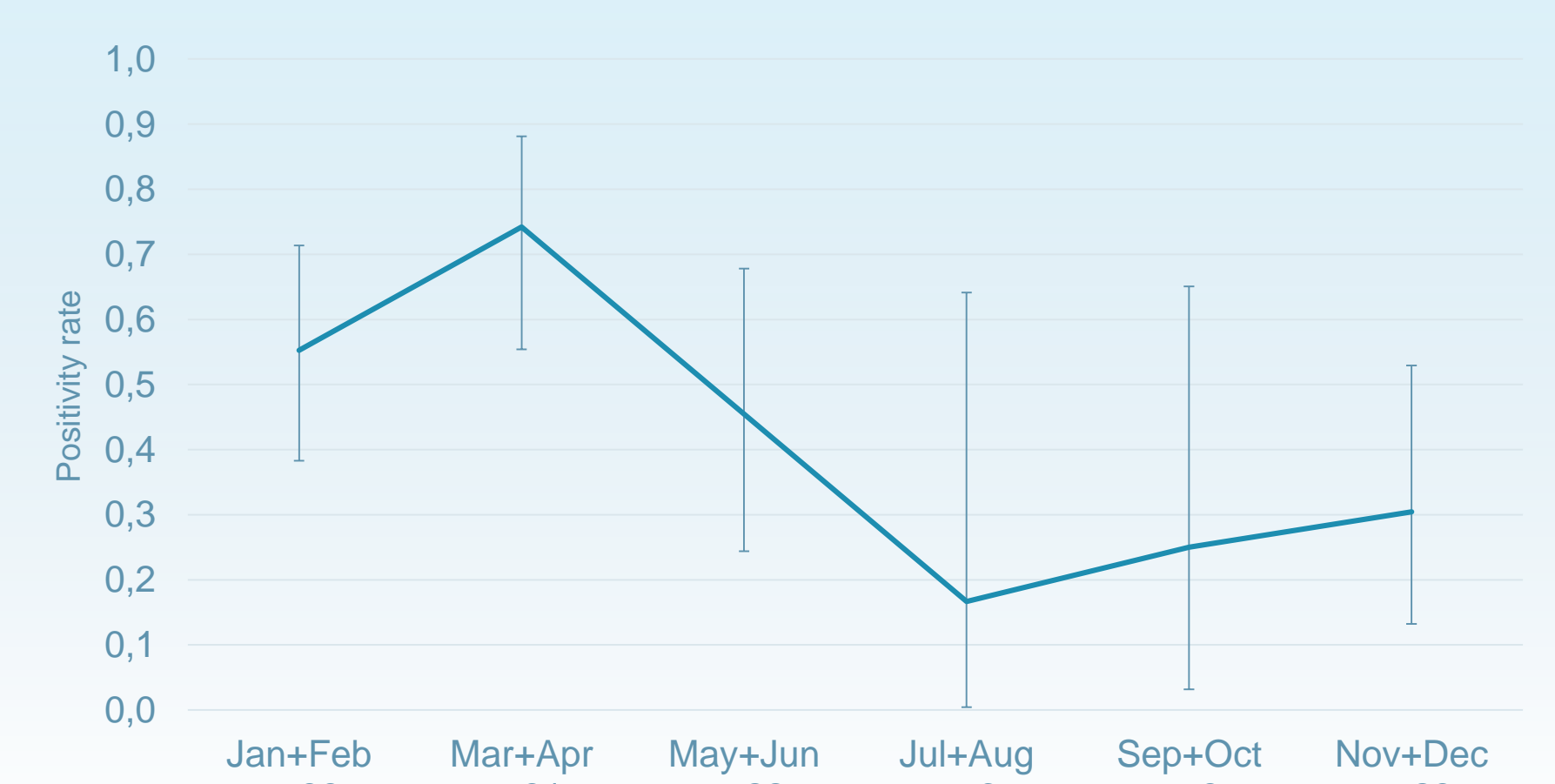
Overall positivity rate of *Pneumocystis jirovecii* in air samples per setting



- High positivity rates in samples from nurseries and healthy young adults.
- Contrasts with the results from schools and nursing homes.
- Staff in nursing homes were obliged to wear face masks.
- Differentiation in nurseries between contribution to shedding from children versus caregivers was impossible.

Year-round positivity rate of *Pneumocystis jirovecii* in air samples from nurseries

- *Pneumocystis jirovecii* DNA year-round detected in the nursery.
- Trend to higher positivity rates during the first 4 months of the year, with a decline thereafter.
- For some periods insufficient data to draw clear-cut conclusions on seasonal variation.



Conclusions

Our results highlight the importance of *Pneumocystis jirovecii* shedding by minimally symptomatic or asymptomatic carriers, particularly infants, toddlers, and young adults. Our findings suggest that immunocompromised patients may be exposed to *Pneumocystis jirovecii* in daily life more frequently than expected, encouraging the use of face masks and sufficient ventilation. Further, we seek to open the debate on monitoring exposure to respiratory pathogens in specific medical settings, such as intensive care units or haematology wards. Environmental air sampling may be a suitable tool for this purpose.