Assessment of SARS-CoV-2 and other IAQ parameters in 11 Belgian elderly care homes

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ABSTRACT

It is often difficult to implement prevention recommendations and plan targeted measures to limit the spread of airborne viruses in communal spaces. To effectively accomplish this goal, it is crucial to comprehensively characterize the indoor environmental quality in the space and, from these space-specific data, draw recommendations adapted to the setting. In this context, 11 elderly care homes in Belgium were selected for a comprehensive assessment of the indoor air quality (IAQ). IAQ and ventilation parameters were characterized by means of air sampling and questionnaire application. In each elderly care home, a survey on ventilation strategies and behaviour, building characteristics and COVID-19 prevention practices was applied, and 5 rooms were selected for IAQ measurements (2 resident bedrooms, at least one common room for residents, one staff room and one extra common room for residents or visitors). In each room, a set of IAQ parameters expected to be related to the indoor virus transmission (CO_2, temperature and relative humidity in all rooms, plus $PM_{2,5}$ in selected rooms) were continuously monitored with an in-house developed and calibrated sensor box for 7 days. Biological samples were collected from the air (via liquid impingement) and surfaces (via swipe sampling), once per room, for in-lab detection of SARS-CoV-2 RNA copies via RT-qPCR. Particulate matter ($PM_{2,5}$) concentrations in all facilities were most of the time low, not exceeding the applicable $PM_{2.5}$ indoor guideline value. CO_2 concentrations generally indicated acceptable levels of ventilation in all facilities, with the lowest CO_2-concentrations measured in mechanically ventilated ones. Of all collected air samples, 28% contained traces of SARS-CoV-2 RNA, while 63% of the swab samples did. The number of positive SARS-CoV-2 samples collected in each elderly care centre did not follow the trend of Flemish COVID-19 incidence rate during each respective sampling week. In the care homes where there was an on-going COVID-19 outbreak during measurements, all air samples and most of the surface samples tested positive for SARS-CoV-2. In the absence of a reported on-going local outbreak, positive SARS-CoV-2 samples were found mainly on surfaces. These results indicate that more than one positive SARS-CoV-2 air sample in one building might work as an indicator of an on-going outbreak (or at least of the presence of SARS-CoV-2 RNA emitters), which in the context of asymptomatic persons in this setting occupied by a vulnerable population is of high value. Furthermore, even though an indoor CO_2 concentration below 900 ppm is often considered indication of lower risk of indoor virus transmission, the dominant variant at that time of the pandemic (delta) appeared in all SARS-CoV-2 positive air samples. Therefore it is recommended to initiate additional risk reduction strategies in case of a local outbreak, such as increasing ventilation rates, implementing effective air cleaning, using mouth masks and isolating infected persons (symptomatic or not), especially in sensitive settings like these.

KEYWORDS

Indoor air quality, elderly care homes, airborne pathogens, SARS-CoV-2, COVID-19 prevention

1 INTRODUCTION

Respiratory viruses spread mostly through the air, both in short and long ranges, especially in crowded and/or poorly ventilated closed spaces (Morawska and Cao, 2020; Abdin and Mahmoud, 2024). Due to the lack of solid information about indoor air quality (IAQ) and ventilation/airing facilities and behaviour in communal spaces, especially those occupied by vulnerable populations such as children and the elderly, it is often difficult to formulate targeted recommendations for infection prevention adapted to the setting and its actors. A comprehensive characterization of such indoor environments and their IAQ is crucial to generate appropriate preventive guidelines and recommendations adapted to the setting, based on space-specific data, relevant information and scientific evidence (Kakoulli et al., 2022).

In this context, different communal spaces in Belgium, including spaces for susceptible population groups, were selected for a comprehensive characterization of the indoor environment by means of IAQ measurements and a dedicated questionnaire on ventilation/disinfection behaviour of relevant actors. The data presented in this paper are part of a larger scale Belgian study during the COVID-19 pandemic in 2022, the "AIR-CO" project, which targeted IAQ assessments in elderly care centres, schools, indoor sports facilities and public transport. The primary aim of this study was to provide relevant, space-specific and up-to-date information on IAQ in a range of representative communal spaces to assist in the formulation of appropriate preventive recommendations and guidelines in the context of pandemic preparedness. The present paper focuses on part of this study, reporting the data collected in the elderly care homes in comparison to relevant IAQ Belgian guidelines (Flemish Indoor Environment Decree and Recognition Standard of Residential Care Centres).

The endpoint of this study was to identify priority risk environments and/or parameters to prioritize environments and to help formulate specific solutions or recommendations for a dedicated risk reduction in a specific setting. The ultimate goal was to contribute to the preventive approach to the spread of respiratory viruses in the future, particularly regarding COVID-19 and current/future variants. Finally, the findings can be extended to other sensitive settings in which people come together for relatively long periods of time, e.g. daycare centres, multi-purpose rooms, catering establishments, shopping centres.

2 MATERIAL AND METHODS

2.1 Sampling locations

In total, 11 elderly care homes (or WZCs, from the Dutch *woonzorgcentra*) across Belgium were selected. Indoor assessments took place during the first half of 2022. In each elderly care home (WZC), 5 rooms were selected for measurements: 2 resident bedrooms, at least one common dining room for residents, one staff room and one extra common room either for residents or for visitors, where available. In each selected room, indoor IAQ parameters (CO_2, temperature and relative humidity in all rooms, plus $PM_{2,5}$ in selected rooms) were continuously monitored with an in-house developed sensor box for 7 consecutive days. During this period, air and surface biological samples were collected once per room to determine the presence of SARS-CoV-2 via qPCR analysis.

2.2 Measurement techniques

Custom-made monitoring devices (sensor boxes) containing various low-cost electronic sensors were built for this study to continuously monitor indoor temperature (T), relative humidity (RH), CO_2 and particulate matter ($PM_{2.5}$) in the selected rooms, at a 1-min time resolution. An integrated internal memory allowed secure storage of the measured data during the measurement periods. All sensors integrated into the sensor box were calibrated in lab prior to the assessments, against reference gases/mixtures under controlled conditions (test chamber) using standard measuring instruments as references. The installation of the sensor boxes and practical organisation of the IAQ assessment respected the ISO 16000-1 standard.

Biological samples for viral pathogen analysis were collected in each assessed room on the same day the sensor boxes were installed, in two different formats: surface samples via swabbing and air samples via liquid impingement. Swabs were collected using 3M™ hydrated sponges. Just before sampling, the liquid was poured onto the sponges, which were then rubbed on the desired surface for a few seconds. Ventilation grilles were the primary target surfaces in each room as they represent known points of particle accumulation. High-touch surfaces were the secondary targets (e.g. doorknobs, handles, tabletops, armrests of chairs). The entire target surfaces were scrubbed, thus the surface areas varied considerably between swab samples.

Airborne viral particles were sampled using a Coriolis µ device manufactured by Bertin Technologies (St-Berthely, France), consisting of a cyclonic liquid impinger that captures aerosols with an aerodynamic diameter between 0.5 and 20 µm at high air flow rates. Air sampling was performed according to Paralovo et al. (2024): 3 m^3 of air sampled in 3 ml of lysis buffer with one droplet of anti-foaming agent.

One air sample and three swab samples were collected in each assessed room of each selected WZC. The placement of the Coriolis µ in the room also respected ISO 16000-1. After sampling, all biological samples were analysed in lab via RT-qPCR analysis, following the protocol described by Janssens et al. (2022). The test targeted three SARS-CoV-2 gene fragments: the nucleocapsid (N1 and N2) and the virus envelop.

Lastly, a survey was conducted to collect relevant information about the selected WZCs, focusing on ventilation characteristics and behaviour as well as disinfection practices. The responses were obtained from the person available to host the research team at the WZC (either a facility manager or a designated nurse).

3 RESULTS AND DISCUSSION

3.1 Facilities characteristics and questionnaire responses

Table 1 summarizes the most relevant information collected in the responses to the questionnaires (3 of the 11 WZCs did not respond to the questionnaire). Out of the 11 WZCs, 7 had a balanced mechanical (type D) ventilation system, 3 had only natural ventilation (i.e. ‘none’ in Table 1), while WZC1 consisted of a newer block with mechanical ventilation (type C: natural air supply and mechanical exhaust) and an older block with only natural ventilation.

Table 1: Most relevant information collected in the responses to the questionnaires.

	WZC										
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Number of rooms	81	100	96	94	67	93	-	90	114	-	-
Number of staff	80	92	90	67	50	57	-	80	90	-	-
COVID-19 vaxx staff	≥90%	≥90%	≥90%	80-90%	≥90%	≥90%	-	≥90%	≥90%	-	-
COVID-19 vaxx residents	≥90%	≥90%	≥90%	≥90%	≥90%	≥90%	-	≥90%	≥90%	-	-
COVID-19 booster residents	≥90%	≥90%	≥90%	≥90%	≥90%	≥90%	-	≥90%	≥90%	-	-
Number of residential units	3	3	3	2	3	3	-	4	3	-	-
Construction year	<2000	<2000	>2000	<2000	>2000	>2000	-	>2000	>2000	-	-
Infection prevention committee?	Yes	Yes	Yes	No	No	No	-	Yes	No	-	-
Cleaning surfaces: high-touch surf.	Daily	Daily	Daily	Daily	Daily	Daily	-	Daily	Daily	-	-
Cleaning surfaces: not touched often	Weekly	Weekly	Weekly	GA	Daily	Weekly	-	Monthly	Weekly	-	-
Isolation of COVID+ residents	Ward/ Room	Room	Room	Room	Ward	Room	-	Room	Ward/ Room	-	-
Type mech. vent. system*	C / None	None	D	None	D	D	D	D	D	D	None
Ventilation altered since COVID-19	No	No	Yes	No	No	No	-	Yes	No	-	-

*Information collected by the research team regardless of survey response.

Overall, it was noticed that several facilities had difficulty answering questions regarding the ventilation systems and their practical operation in the facilities. This observation is in line with research projects on ventilation and IAQ performed by the research team in the last 15 years, e.g. in a study on the IAQ and ventilation characteristics of 15 Flemish WZCs (Flemish Government, 2024) and in several IAQ assessments in Flemish schools and daycare centres (De Jonge et al., 2023; Paralovo et al., 2023). This highlights the need for further sensibilization among WZC staff in general regarding the importance of ventilation.

3.2 CO_2 and $PM_{2,5}$

During the COVID-19 pandemic a concentration of 900 ppm CO_2 (or 40 m^3 $person^{-1}$ $hour^{-1}$) was recommended in Belgium as an indicator of sufficient ventilation in any indoor environment. This recommendation was exceeded at least once in at least one of the assessed rooms in all WZCs studied. Regarding $PM_{2.5}$, all WZCs showed high isolated concentration peaks (most likely related to indoor activities such as cleaning), but during most of the sampling time concentrations were considerably low, complying with the Indoor Air Guideline's target value of 10 µg m^{-3} as specified in the Flemish Indoor Air Decree (Flemish government, 2018), which applies to the public as well as private rooms in a WZC. Although low-cost sensors in general have lower accuracy than other IAQ monitors, their generated data are highly useful if they are properly calibrated.

Figure 1 summarizes the CO_2 and $PM_{2,5}$ data measured during the occupied hours in boxplots, in two different categorizations: by type of room (resident bedroom, common room, staff room and others) and by the presence of mechanical ventilation in each room. The occupied hours were defined per room type as:

- Common areas: 7h30 to 9h30 (breakfast), 11h to 13h (lunch), 16h30 to 18h30 (dinner)
- Resident rooms: 20h to 7h (sleeping hours)
- Staff rooms and others: 8h to 18h (working hours)

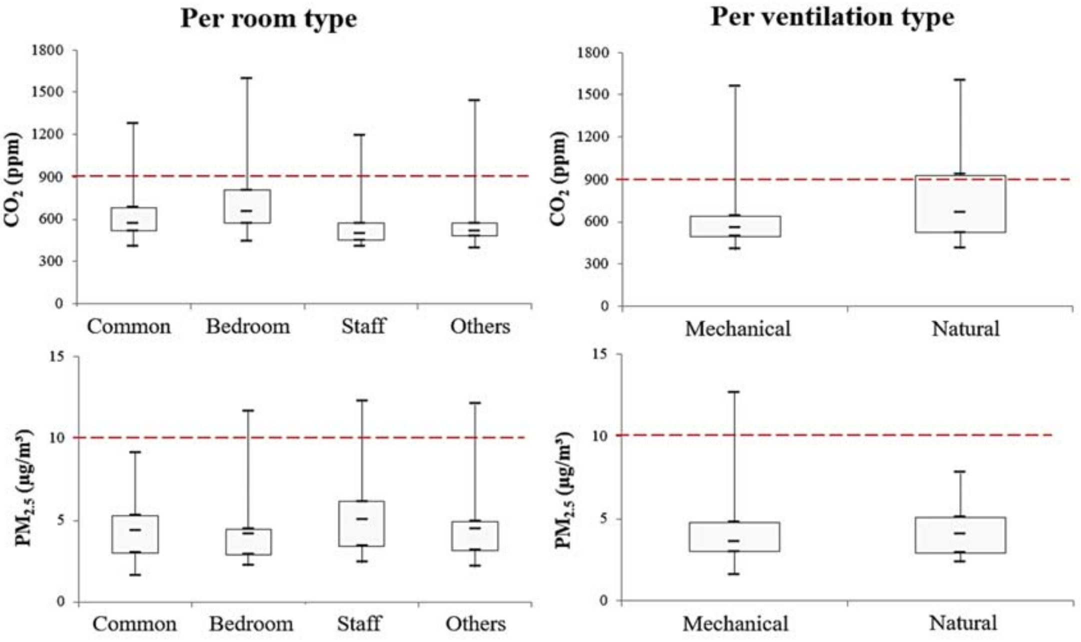

Figure 1: Concentrations of CO_2 (top) and $PM_{2.5}$ (bottom) classified by room type (left; 'others' include all assessed areas that have a different use: visitors cafeteria, entrance hall, hair salon, physiotherapy room) and type of ventilation in the room (right).

Analysing the box plots on the left side of Figure 1, no striking differences can be noted between the different room types in terms of CO_2 or $PM_{2.5}$ levels. It appears that resident bedrooms achieve slightly higher CO_2 concentrations, while staff rooms and others have the lowest overall concentrations on a daily basis (which is expected due to lower and more variable occupancy in the latter). The higher CO_2 concentrations in some resident rooms may indicate a less adequate ventilation during the night, which is commonly noticed in bedrooms as during the night, people tend to close windows and doors and ventilation systems often have a lower flow rate for energy saving reasons. The opposite was observed for $PM_{2.5}$: the resident bedrooms had the lowest concentrations. This was expected because events that usually suspend the most particles to the air (e.g. dusting, cleaning, occupant's movement) usually occur during the working hours, which were excluded from the bedrooms' datasets for this analysis.

The box plots on the right side of Figure 1 indicate a clear difference between the CO_2 levels for the mechanically ventilated rooms versus the non-mechanically ventilated rooms. Although the maximum values were very similar, the mechanically ventilated rooms showed considerably lower P75 and average CO_2 concentrations, while in the naturally ventilated rooms the CO_2 concentration was above 932 ppm for 25% of the occupied period. Only in the 4 WZCs without mechanical ventilation (WZCs 1, 2, 4 and 11) did the P75 value exceed the 900-ppm guideline in at least one of the investigated areas. On the other hand, $PM_{2.5}$ concentrations were only moderately lower in the mechanically ventilated WZCs. A possible explanation is that most WZCs tend to be located in areas where outdoor PM is also considerably low (i.e. away from urban centers), and thus the absence of filtration of incoming air by mechanical ventilation systems might not be as relevant regarding the indoor PM.

3.3 Temperature and RH

According to the Flemish Indoor Environment Decree, indoor T must remain between 20-24°C and indoor RH between 40-60% during the cold season, and between 22-26°C and 30-70% during the warm season. Specifically for WZCs, the Decree of the Flemish Government regarding the Recognition Standard of Residential Care Centres (2019) stipulates that T in all living areas during the day is at least 22°C regardless of the season, and that all measures are taken to maintain $T < 26°C$ in all accommodation spaces. Table 2 provides an overview of T and RH values measured in the 11 WZCs during occupied hours. Cells are coloured according to the Flemish recommendations: orange cells indicate $T > 28°C$ or $RH < 30\%$, yellow cells indicate $28°C > T > 26°C$ or $30\% < RH < 40\%$, dark blue cells indicate $T < 20°C$ or $RH > 70\%$, and light blue cells indicate $20°C < T < 22°C$ or $70\% > RH > 60\%$.

In addition to its contribution to the thermal comfort of occupants, T can also strongly affect their behaviour, especially regarding ventilation (e.g. windows opening). Table 2 shows that in most WZCs there was a trend towards temperatures above the recommended value (26°C), even though the measurements were carried out during the cold season. WZC3 was particularly warm, as even the minimum temperatures in both resident bedrooms were above the maximum recommendation. This tendency towards overheating was less apparent in the rooms where residents are not expected to be present (staff rooms and others). On the other hand, in these rooms (and less often in the resident bedrooms and common areas) there were also some cases where T fell below the minimum recommended value for WZCs (22°C) or even the value stipulated by the Flemish decree (20°C).

Various of the interviewed employees reported that the residents often complain about feeling cold or chilly at temperatures that the staff themselves find pleasant, and that they therefore often increase heating in the resident rooms and common areas. Some employees also mentioned that they often avoid opening windows, even if they feel that the air is "stuffy", for the sake of residents' thermal comfort.

Table 2: Overview of the temperature and RH measured during occupied periods in each studied room per WZC.

		T [°C]											RH [%]										
WZC		1	2	3	4	5	6	7	8	9	10	11	1	2	3	4	5	6	7	8	9	10	11
1st Res. bedroom	Max.	24,2	25,2	29,3	27,4	27,7	27,4	26,5	26,1	27,7	27,0	26,2	44,8	30,5	29,8	30,4	38,5	39,1	36,2	34,1	30,6	39,7	49,4
	P75	22,5	24,7	29,0	26,7	25,3	26,0	26,1	24,9	26,9	26,3	23,9	36,2	26,6	27,3	21,9	31,5	32,7	31,3	31,8	25,7	37,1	46,1
	Mean	21,7	24,4	28,7	25,4	24,5	25,6	25,8	24,0	25,7	26,0	23,5	33,0	25,6	21,8	19,7	29,0	30,4	28,5	26,7	23,1	34,7	43,4
	Min.	19,1	22,9	27,4	22,9	22,6	24,2	25,0	19,0	23,4	25,1	22,4	22,6	22,2	13,6	15,4	22,0	20,0	24,3	16,3	16,2	26,9	29,8
2nd Res. bedroom	Max.	22,0	24,9	29,4	27,1	30,2	25,2	26,5	25,6	-	-	28,7	54,0	34,4	31,5	32,8	36,2	-	37,5	35,4	-	-	47,2
	P75	21,2	23,9	28,5	26,3	27,8	24,4	26,0	24,6	-	-	25,9	49,3	32,7	28,0	31,2	28,1	-	30,5	32,4	-	-	42,9
	Mean	20,5	23,5	28,1	25,9	26,9	24,1	25,4	24,3	-	-	25,1	46,4	30,8	22,5	25,6	26,1	-	27,8	28,3	-	-	39,4
	Min.	19,3	22,2	26,5	24,7	24,4	23,0	24,1	23,2	-	-	23,2	38,0	25,9	14,9	16,0	19,0	-	21,4	22,5	-	-	27,2
1st Comm. room	Max.	27,9	25,2	27,8	32,0	26,4	26,2	25,8	25,8	25,6	32,3	-	43,1	31,4	36,7	43,6	61,9	42,2	42,5	43,5	38,2	49,1	-
	P75	26,2	24,3	26,3	24,5	24,4	25,5	25,1	24,8	24,8	28,6	-	34,2	27,2	30,0	31,9	32,3	33,5	33,0	31,8	27,8	35,4	-
	Mean	24,9	23,6	25,5	23,6	23,9	25,1	24,7	24,1	24,4	27,9	-	30,4	25,3	24,8	27,4	29,2	29,8	29,7	27,9	24,8	32,6	-
	Min.	20,4	20,4	17,8	19,3	21,3	23,3	23,0	21,7	19,8	23,2	-	20,1	19,1	14,5	13,1	19,2	14,9	22,6	18,9	15,5	22,6	-
2e Comm. room	Max.	24,6	-	-	-	-	-	-	27,1	27,0	-	-	41,5	-	-	-	-	-	-	37,1	37,8	-	-
	P75	23,7	-	-	-	-	-	-	25,9	26,2	-	-	34,2	-	-	-	-	-	-	28,2	26,8	-	-
	Mean	23,4	-	-	-	-	-	-	25,3	25,8	-	-	31,3	-	-	-	-	-	-	25,2	24,6	-	-
	Min.	21,9	-	-	-	-	-	-	22,6	24,1	-	-	20,7	-	-	-	-	-	-	17,1	16,6	-	-
Staff room	Max.	24,0	22,9	25,0	27,0	26,2	27,5	25,1	22,3	25,6	23,8	28,0	52,8	58,4	43,7	32,3	44,1	39,6	58,6	44,9	41,4	57,0	49,4
	P75	22,9	19,1	23,7	26,7	25,5	26,0	24,1	21,3	24,3	23,2	26,8	41,3	30,4	32,7	27,8	32,5	32,1	34,2	32,8	31,3	46,9	41,1
	Mean	22,2	18,4	23,3	26,2	24,8	25,5	23,8	20,1	23,4	22,6	26,4	36,8	28,6	27,8	24,5	29,3	28,7	31,6	29,7	27,3	40,6	37,1
	Min.	18,5	15,5	18,5	24,8	21,4	21,8	22,6	18,2	19,6	20,7	24,7	18,9	21,4	17,0	16,1	16,3	16,4	25,7	20,7	18,6	23,0	28,1
Others	Max.	-	21,9	25,2	25,2	33,7	24,5	26,7	-	25,1	25,9	27,6	-	34,9	34,8	39,0	43,7	50,5	36,0	-	43,6	53,9	45,6
	P75	-	21,2	24,5	24,7	23,8	23,9	25,1	-	24,4	24,3	26,8	-	31,8	31,8	30,9	34,3	37,1	33,6	-	30,6	41,8	38,4
	Mean	-	20,8	24,2	24,6	23,2	23,7	24,4	-	23,9	24,0	26,3	-	29,4	26,4	27,5	30,2	32,7	31,4	-	27,1	37,1	33,3
	Min.	-	17,7	23,2	23,9	19,9	21,4	22,9	-	21,3	21,5	22,9	-	25,2	17,0	18,8	12,1	19,8	26,9	-	18,4	24,4	21,1
Outdoor	Max.	13,0	13,0	16,0	16,0	18,0		19,0	17,0	17,0	18,0		100	88	87	87	93		100	93		100	
	P75	9,0	8,0	12,0	12,0	11,0		15,0	9,0	8,0	13,0		82	72	62	71	81		76	81		87	
	Mean	5,8	5,4	8,2	8,2	8,3		10,7	5,9	5,9	10,5		74	62	50	58	69		61	72		74	
	Min.	-1,0	-2,0	-4,0	-3,0	0,0		0,0	-2,0	-2,0	4,0		40	36	23	28	23		24	39		25	

RH was generally very low in most of the rooms examined in the 11 WZCs. It was particularly dry in the resident bedrooms, since in all but two WZCs (WZC1 and WZC11) even the maximum RH achieved in the resident bedrooms was below the recommended minimum for the cold season (40%). In many of the studied rooms, the P75 and average values were below the recommended minimum RH even for the warm season (30%). RH tends to decrease during the heating season, especially in buildings with mechanical ventilation where incoming air is heated. However, RH did not seem to be influenced by the presence of a mechanical ventilation system in this study.

Besides being an important comfort parameter, RH is also an important point of attention to limit the transmission of viruses indoors. Evidence from various international studies indicates that dry airways make people more susceptible to airway infections (Courtney and Bax, 2021). It has also been shown that humidity influences both evaporation kinematics and particle growth, meaning that in dry indoor spaces (< 40% RH) the risk of airborne transmission of SARS-CoV-2 is greater than that of humid spaces (Ahlawat et al., 2020). Recent research indicates a strong negative relationship between RH and the transmission of both SARS-CoV-2 and influenza (Keetels et al., 2022), partly due to the greater sensitivity of respiratory tracts at lower humidity.

3.4 Viral particles

Table 3 presents the results collected after qPCR analysis of each biological sample (from the air and from the surfaces) collected in the 11 WZCs. It also indicates whether there was an active (A) or recent (R, i.e. active in the week prior to assessment) COVID-19 outbreak in the facility (N indicates neither active nor recent outbreaks reported). The facility with the highest percentage of positive samples was WZC10 (where only one of the samples collected, both in air and on surfaces, was negative), and the facility with the lowest percentage was WZC1. No clear correlation between positive viral samples and the other IAQ parameters measured was observed. The data shown in Table 3 clearly indicates a direct relation between an on-going COVID-19 outbreak and a high frequency of SARS-CoV-2 detection, in any room, both in air and on surfaces, which reflects the complexity of isolating infected persons to prevent virus transmission in a WZC during an outbreak; even when other IAQ parameters (i.e. CO_2 and $PM_{2,5}$ concentrations) indicate sufficient ventilation.

Table 3: Presence of SARS-CoV-2 in each biological sample taken in each room examined per WZC.

		SARS-CoV-2 (Ct-value)										
WZC		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
1e Resident bedroom	**Air**	Neg	Neg	Neg	Neg	Neg	Neg	34,5	Neg	Neg	28,9	-
	Surf. 1	36,2	34,9	32,8	Neg	Neg	32,7	Neg	31,6	Neg	30,3	33,3
	Surf. 2	Neg	33,4	35,5	35,9	44,9	Neg	Neg	29,7	Neg	26,4	34,7
	Surf. 3	Neg	Neg	35,9	Neg	Neg	35,9	Neg	34,0	Neg	28,5	33,5
2e Resident bedroom	**Air**	Neg	Neg	Neg	Neg	Neg	-	-	Neg	-	33,3	35,5
	Surf. 1	Neg	33,8	Neg	Neg	Neg	Neg	Neg	32,2	-	33,8	31,7
	Surf. 2	Neg	34,5	Neg	35,7	35,6	Neg	Neg	30,4	-	30,9	34,9
	Surf. 3	Neg	36,0	36,3	Neg	Neg	Neg	34,0	27,0	-	31,8	Neg
1st Common room	**Air**	Neg	Neg	Neg	Neg	35,2	Neg	Neg	Neg	Neg	30,6	33,8
	Surf. 1	34,6	33,8	34,9	Neg	34,0	33,4	Neg	30,7	35,3	35,1	36,9
	Surf. 2	Neg	34,5	34,8	35,6	31,2	33,2	35,6	34,8	33,9	34,1	Neg
	Surf. 3	Neg	36,0	Neg	Neg	29,4	Neg	32,7	33,0	Neg	28,0	33,8
2nd Common room	**Air**	Neg	-	-	-	-	-	-	Neg	34,2	-	-
	Surf. 1	32,6	-	-	-	-	-	-	37,9	27,7	-	-
	Surf. 2	35,6	-	-	-	-	-	-	33,4	27,9	-	-
	Surf. 3	Neg	-	-	-	-	-	-	32,0	31,8	-	-
Staff room	**Air**	Neg	Neg	Neg	Neg	32,7	Neg	Neg	Neg	-	31,1	33,4
	Surf. 1	37,2	Neg	35,8	36,2	Neg	Neg	Neg	38,2	Neg	37,0	33,2
	Surf. 2	35,0	34,8	Neg	Neg	35,1	Neg	33,2	34,7	Neg	Neg	Neg
	Surf. 3	38,8	35,6	35,8	36,7	34,6	Neg	35,0	32,0	Neg	36,9	Neg
Other	**Air**	-	Neg	Neg	35,1	Neg	Neg	Neg	-	Neg	36,0	36,6
	Surf. 1	35,9	33,8	Neg	Neg	35,2	38,2	34,2	-	Neg	34,4	34,7
	Surf. 2	Neg	36,9	34,6	32,8	Neg	33,4	32,9	-	30,5	36,4	Neg
	Surf. 3	-	36,0	33,8	33,1	Neg	38,0	35,7	-	Neg	36,2	Neg
Rate of positives (%)		36	65	50	40	53	37	47	75	37	95	68
Covid-19 outbreak?		N	R	N	N	N	N	N	R	N	A	A

Table 3 also presents the cycle threshold (Ct) values for each of the samples in which SARS-CoV-2 was detected. In a qPCR test, the Ct value is inversely proportional to the amount of viral RNA contained in the sample (Paralovo et al., 2024). The qPCR test stops after Ct = 45. The method's LoQ was determined as 20 gene copies/ml of liquid sample (Janssens et al., 2022), but Ct values are not converted to RNA copies/ml in this study because it was not possible to generate a standard calibration curve for SARS-CoV-2 in lysis buffer at that time.

Although the Ct value is not always a measure of infection risk, as the genetic target of both viable and non-viable microorganisms is measured indiscriminately, it might be considered as a proxy for infection risk, especially when applied to environmental samples (i.e. from the air and surfaces), as opposed to those taken from human body fluids (i.e. not yet distributed to the environment and thus not at immediate risk of contact with other individuals).

On the other hand, environmental samples will normally contain much smaller amounts of viral RNA compared to human samples, regardless of the concentration of virus-laden bioaerosols in the environment assessed, since the bioparticles emitted by infected individuals spread throughout the air volume in the room they are in, leading to high dilution factors. This means that the Ct value thresholds commonly considered to determine whether a sample is positive for e.g. nasal swabs (usually Ct value < 30) are not as adequate for environmental samples. Therefore, higher Ct values should still be considered indicative of a positive environmental sample. Paralovo et al. (2024) suggests that Ct values lower than 39.3 still offer a significant chance of being configured as positive air samples for SARS-CoV-2, while Ct values above 39.3 have greater uncertainty and therefore a smaller chance of configuring a true-positive.

In Table 3, the cells are coloured according to the Ct value of the sample: red for $Ct < 30$ (i.e. clearly positive, in the same range as nasal swabs), orange for $30 < Ct < 35$ (i.e. smaller viral load but higher chance of configuring of true positive), yellow for $35 < Ct < 39.3$ (i.e. a very small viral load, lower probability of configuring true positive) and green for $Ct > 39.3$ (samples considered negative for SARS-CoV-2). It is also important to note that identifying environmental samples as positive for SARS-CoV-2 RNA provides only a quick but superficial assessment of the potential infectivity in a given area (i.e. not a measure of viable virions).

Figure 2 shows the evolution of COVID-19 new cases per week among the Flemish population between February and April 2022, when the measurements were performed (Sciensano, 2024).

Figure 2: Weekly incidence of COVID-19 in the Flemish population during the entire sampling period.

An initial hypothesis in this study was that the incidence of COVID-19 in the WZCs (and therefore the number of positive biosamples) would (at least loosely) follow the regional or national incidence (both followed a very similar trend in the same period). If that were the case, WZC7 would have the highest number of positive samples, and the simultaneously sampled WZCs would have similar rates. Yet, analysing Table 3, this hypothesis is not confirmed: the highest percentage of positive samples was collected when the Flemish incidence was at relatively low level and pairs of simultaneously sampled WZCs differed considerably. The most likely explanation was that the WZC sample size was too small to observe such trend. The highest number of positive samples was taken in WZC10. The facilities with the 2nd and 3rd highest numbers of positive samples were WZC8 and WZC11. However, in WZC8 only swab samples were positive, while in WZC11 all four air samples collected were positive.

The measurements in WZC10 and WZC11 took place simultaneously in April 2022, while active COVID-19 outbreaks were occurring in both facilities. This is clearly reflected in the air sample results: WZC10 and WZC11 were the only facilities where all air samples collected were positive. An interesting situation occurred in WZC8, where the research team was informed by staff that a COVID-19 outbreak was ongoing during the measurement week. An outbreak had already occurred there a week before the measurements started, but some residents tested positive again and the staff therefore assumed that the outbreak was still ongoing. However, at the end of the measurement week, the staff informed the research team that it was indeed a 'false alarm', i.e. that on a second test all previously infected residents showed a negative result. This was also reflected in the WZC8 results: all air samples were negative, and all surface samples were positive, indicating a facility-wide outbreak in the past, but that was no longer active. A similar situation occurred in WZC2, where the staff reported that a COVID-19 outbreak had ended in the week prior to sampling. As shown in Table 3, all air samples in WZC2 were negative, while all but two surface samples were positive.

In fact, only 28% all air samples collected in WZCs were positive, while 63% of all swab samples were positive. Indeed, it was expected that more positive swab samples than air samples would be found due to the bioaerosol cycle itself: aerosols containing pathogenic material are emitted into the air by infected individuals remain suspended in the air for a period (depending on their particle size), and then settle by gravity on the inner surfaces. Deposition of such particles can also happen on high-touch surfaces by e.g. contaminated hands. In general, viral bioaerosols can remain airborne for a few hours, but once settled these particles can persist for 1 to 28 days (Suman et al., 2020; Marzoli et al., 2021), depending on the type of surface, the cleaning regime and the RNA degradation rate by RNAses present in the environment. It is therefore reasonable to consider the presence of airborne viral aerosols as a possible indication of an active emission source (e.g. during an ongoing COVID-19 outbreak or at least the presence of an infected person/emitter during sampling), while the presence of viral material on the surfaces could indicate a past emission source that may no longer be active (e.g. traces of SARS-CoV-2 remaining on surfaces several days or weeks after the end of a COVID-19 outbreak, depending on the 3 factors mentioned above). It is, however, important to keep in mind that the presence of an emitter of SARS-CoV-2 RNA does not necessarily implicate on active COVID-19 case(s), since it is possible to emit RNA but not viable virus (e.g. emitted RNA could consist of degraded products from an immune response).

4 CONCLUSIONS

The CO_2 concentrations measured in this set of 11 WZCs generally seem to indicate an acceptable level of ventilation, especially for those with mechanical ventilation systems. Of all collected air samples, 28% contained genomic material, while 63% of swab samples did. The number of positive SARS-CoV-2 samples (from surfaces and air) did not follow the Flemish COVID-19 incidence during the measurement period, possibly due to the small sample size. On the other hand, positive air samples were found in all studied rooms of WZCs undergoing active COVID-19 outbreaks, while positive surface samples seemed to indicate (recent) previous outbreaks. This finding underlines the potential value of a combined assessment of the viral load in air and surface samples as an important tool to screen indoor spaces on the actual infection risk of occupants, without collecting human samples of each resident. Including more pathogens in similar studies would allow a wider assessment of the exposure and risk of susceptible populations, such as the elderly, and would enable a more targeted anticipation on initiated outbreaks. General recommendations for WZCs regarding airborne-spreading infections include more frequent testing of residents and staff (symptomatic or not), isolation of positive-testing residents, use of mouth masks and increased ventilation/airing, especially in communal areas.

Additionally, an overall unawareness regarding ventilation systems was observed in the WZCs studied in this research. While management and staff did demonstrate understanding the importance of airing and ventilating the common spaces, it was very common that they did not know even if their respective facilities had a mechanical ventilation system installed. When they reported that they did have a ventilation system, they often did not know which type. In a few facilities, incorrect ventilation systems were reported, or had its presence/absence misreported. This indicates the importance of further increasing the sensibilization of the public regarding the importance of ventilation, especially when aimed at agents directly responsible for managing spaces where a more vulnerable population is present.

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