PREVALENCE OF ANTI-SARS-COV-2 ANTIBODIES IN THE GENERAL POPULATION IN BELGIUM

RESULTS OF THE SECOND DATA COLLECTION (28/09/2021 – 23/02/2022)

Van den Houte N., Leclercq V., Gisle L. & Van der Heyden J.
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KEY FINDINGS

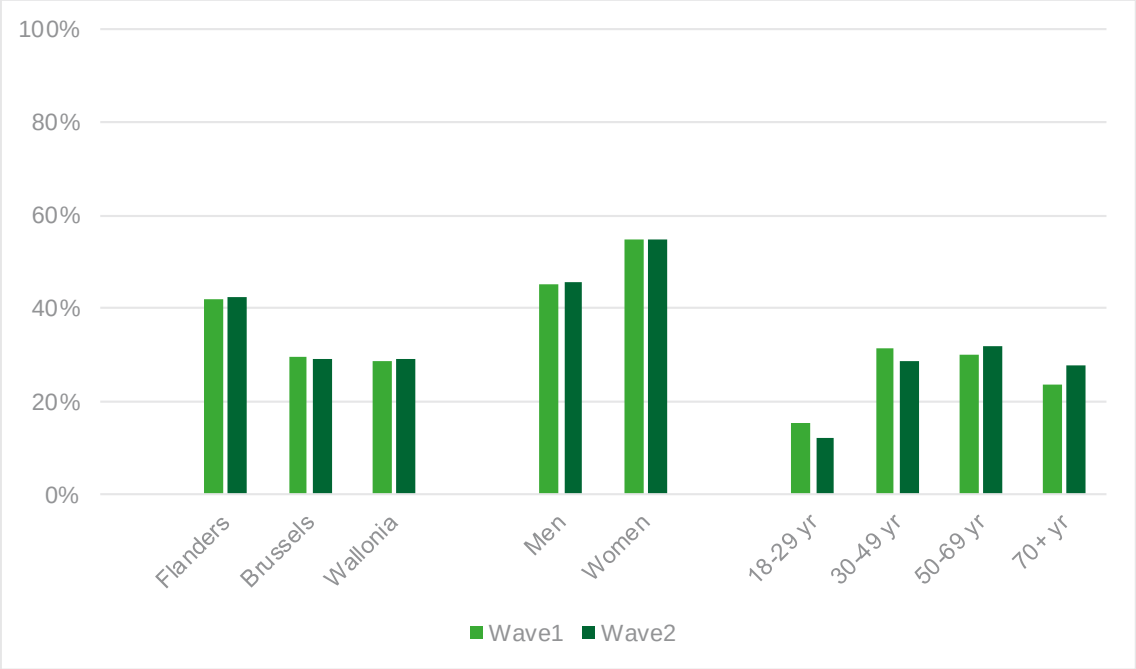
- Between the **last period of wave 1** (14/06-11/07) and the **first period of wave 2** (27/09-24/10) the percentage of adults (18 years or older) with anti-SARS-CoV-2 antibodies has increased **from 69.5% to 90.4%.** This substantial increase is in line with the intensive vaccination campaign during this time period. During the **second period of wave 2** (25/10-21/11) the prevalence increased further to **92.9%.**
- Among the **fully vaccinated having received the last dose (eventually a booster) in the past 3 months (but more than 2 weeks ago),** the prevalence of anti-SARS-CoV-2 antibodies was 97.9% in the first period of wave 2 (27/09-24/10) and 99.1% in the second period (25/10-21/11). Among the **fully vaccinated having received the last dose (eventually a booster) longer than 3 months ago**, these percentages were respectively 93.8% and 95.7%
- Among **people who had not received any COVID-19 vaccine at all** the prevalence of anti-SARS-CoV-2 antibodies increased in wave 2 from **22.5%** (27/09-24/10) to **36.5%** (25/10-21/11). However, caution is needed to interpret these figures, because in wave 2 the number of unvaccinated people was quite low.
- Most of those who had a negative test in wave 1 (87.8%) became positive in wave 2. This is mainly explained by the vaccination campaign: 94.8% of these people received a COVID-19 vaccine between their participation in wave 1 and wave 2. **Only 9.7% of the unvaccinated people with a negative test in wave 1 became positive in wave 2**.
- In total, **3.1% of those who had anti-SARS-CoV-2-antibodies in wave 1 seroconverted in wave 2**. Among the unvaccinated and the partially vaccinated people, the risk of becoming seronegative was 10 times higher than among the fully vaccinated.
- Among the **total study population**, **older age** was associated with a higher risk of not having antibodies in wave 2.
- Among the **fully vaccinated**, **older people** and people with **at least one chronic disease** were at higher risk of not carrying antibodies. The risk of being seronegative in wave 2 increased significantly as the **number of days since the last vaccination** advanced. Furthermore, having received at least one dose of **mRNA vaccine** compared to adenoviral-vectored vaccine reduced the risk of being seronegative by 90%.

1. INTRODUCTION

This second report of the SalivaHIS project focuses on the results of the second data collection wave. Methodological details of the study can be found in the first study report(1). The fieldwork of the second wave took place between 28/09/2021 and 23/02/2022, but 99% of the saliva samples were collected during the months of October, November and December 2021. Of the 2767 participants (18+ years) of the first wave, 1917 also took part in wave 2, which means that there was a 30.7% dropout. As can be seen in Figure 1, the distribution of the sample by region and gender was not very different between wave 1 and wave 2. However, in comparison with wave 1, the dropout was larger among young people than among older people. The time interval between a participant's first and second saliva collection ranged from 73 days to 260 days (2 to 8 months). In the large majority of cases (83.2%) this interval varied between 120 days and 180 days (4 and 6 months). For 1859 participants (97.5%), questionnaire data were available.

The saliva samples of 219 people (11.4%) did not meet the validity criteria for testing and were excluded from analyses. This percentage was lower than in wave 1 (17.6%), but still substantial.

Figure 1 | Distribution (%) of the study sample of wave 1 and wave 2, by region, gender and age group, and data collection wave, SalivaHIS study, Belgium 2022



As applied in wave 1, to mitigate the unbalanced study sample, post-stratification survey weights were calculated based on the population structure at 1 January 2021 obtained from Statistics Belgium as auxiliary database. Also, the clustered design (recruitment of participants within households as primary units) was taken into account in the analyses.

2. VACCINATION STATUS AND HISTORY OF COVID-19 INFECTION

According to the official statistics in Belgium (2), the percentage of fully vaccinated people aged 18 years and above had reached 85.9% by 1 October 2021. By 31 December 2021, this percentage had increased to 87.7% (3). In comparison, the percentage of fully vaccinated people (since over 2 weeks) among the participants of wave 2 was 95.9% (weighted figure). These results show that the vaccination rate in the SalivaHIS study population is higher than that of the official statistics. As is shown in Table 1, this is especially the case in Brussels and Wallonia.

Table 1 | Vaccination rates in the population aged 18 years or older. Comparison between the official statistics and the SalivaHIS study, wave 2, Belgium 2022

	Official statistics 1/10/2021 (fully vaccinated)	Official statistics 31/12/2021 (fully vaccinated)	SalivaHIS wave 2 (fully vaccinated > 2w)
Belgium	85.9%	87.7%	95.9%
Flanders	91.4%	92.7%	98.4%
Brussels	66.4%	72.7%	93.3%
Wallonia	79.8%	83.3%	92.0%

Table 2 provides more detailed information on the SalivaHIS study population in function of the vaccination status. The vaccination status was grouped into four categories, which took into account the time since the last vaccination:

1. fully vaccinated for at least two weeks and having received the last vaccination dose (eventually a booster) in the past 3 months;
2. fully vaccinated for at least two weeks and having received the last vaccination dose (eventually a booster) more than 3 months ago;
3. partially vaccinated (having received at least one dose of vaccine) or having completed the basic vaccination less than 2 weeks ago;
4. unvaccinated.

The table shows that most of the SalivaHIS participants (73.5%) of wave 2 were fully vaccinated since more than 3 months. No distinction is made between people who had received a booster vaccination and only a complete vaccination scheme.

Table 2 | Vaccination status of the SalivaHIS study population (18 years or older) in Belgium and its 3 regions, SalivaHIS study, wave 2, Belgium 2022

	Fully vaccinated (> 2w and < 3m)		Fully vaccinated (≥ 3m)		Partially vaccinated / fully vaccinated < 2 w		Unvaccinated		Total
	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)	Total valid N
Belgium	402	22.4 (19.9-24.9)	1349	73.5 (70.8-76.1)	8	0.3 (0.1-0.6)	76	3.8 (2.7-5.0)	1835
Flanders	178	24.7 (21.1-28.3)	585	73.7 (69.9-77.4)	0	0.0 (0.0-0.0)	12	1.6 (0.6-2.7)	775
Brussels	123	21.9 (17.5-26.4)	374	71.4 (66.3-76.4)	3	0.6 (0.0-1.4)	29	6.1 (3.3-8.8)	529
Wallonia	101	18.3 (14.3-22.3)	390	73.7 (68.9-78.6)	5	0.8 (0.1-1.5)	35	7.2 (4.4-10.0)	531

Table 3 presents information on the percentage of people who reported to have had a confirmed COVID-19 infection. There are important regional differences, with higher percentages of reported COVID-19 infections in Brussels and Wallonia than in Flanders. It should be noted that these results were obtained before the sharp increase of infections due to the Omicron variant (December 2021).

Table 3 | History of reported COVID-19 infection in the SalivaHIS study population (18 years or older) in Belgium and its 3 regions, SalivaHIS study, wave 2, Belgium 2022

	Reported COVID-19 infection < 3m		Reported COVID-19 infection ≥ 3m		No reported COVID-19 infection		Total
	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)	Total valid N
Belgium	178	10.0 (8.1-11.8)	54	2.6 (1.7-3.5)	1650	87.4 (85.4-89.5)	1882
Flanders	49	6.9 (4.7-9.2)	14	1.8 (0.8-2.9)	725	91.2 (88.7-96.7)	788
Brussels	53	11.0 (7.8-14.)	22	5.4 (2.5-8.2)	473	83.6 (79.7-87.6)	548
Wallonia	76	15.1 (11.2-19.0)	18	3.1 (1.5-4.7)	452	81.8 (77.6-86.0)	546

3. PREVALENCE OF ANTI-SARS-COV-2 ANTIBODIES

The SalivaHIS results show that by the third trimester of 2021, 92.9% (95%CI: 91.5%-94.3%) of the population aged 18 years and older had developed anti-SARS-CoV-2 antibodies. This appears to be an overestimation of the overall antibodies' seroprevalence due to the overrepresentation of vaccinated people in the SalivaHIS sample with regard to the official statistics. In Table 4, the prevalence of anti-SARS-CoV-2 antibodies is presented by vaccination status of the population. No results are presented for those who are partially vaccinated or fully vaccinated since less than 2 weeks, because this group is too small (n = 8). It should be noted that during the study period of wave 2 (third trimester of 2021) only 30.4% of the unvaccinated population had anti-SARS-CoV-2 antibodies.

Table 4 | Prevalence of anti-SARS-CoV-2 antibodies in the total study population (18 years or older) by vaccination status, SalivaHIS study, wave 2, Belgium 2022

	Number seropositive / total samples	% (95% CI)
Total study population	1561/1698	92.9 (91.5-94.3)
Fully vaccinated (> 2w and < 3m)	345/357	98.0 (96.7-99.3)
Fully vaccinated (≥ 3m)	1126/1199	94.5 (93.2-95.9)
Unvaccinated	19/65	30.4 (15.9-44.8)

4. PREVALENCE OF ANTI-SARS-COV-2 ANTIBODIES BY SOCIO-DEMOGRAPHIC CHARACTERISTICS

Table 5 gives an overview of the differences in the prevalence of anti-SARS-CoV-2 antibodies in function of socio-demographic characteristics. As mentioned above, the antibody seroprevalence estimates are somewhat overestimated because unvaccinated people are underrepresented in the SalivaHIS wave 2 sample. However, the analysis of the associations between socio-demographic characteristics and anti-SARS-CoV-2 antibodies prevalence remains accurate despite this bias.

The following results (Table 5) concern the total study population, as the number of unvaccinated people was too low to conduct separate analyses by vaccination status. The first column presents the antibody seroprevalence and 95% confidence interval (95% CI) in each socio-demographic category. The crude (binary)

association estimates (Odd Ratios (OR) and 95% CI) are shown in the second column. The next two columns report the associations after adjustment for age and/or sex (column 3) and additional adjustment for vaccination status (column 4).

Table 5 | Prevalence and association of anti-SARS-CoV-2 antibodies and socio-demographic characteristics among the study population (18 years or older), SalivaHIS study, wave 2, Belgium 2022

Variable	Categories	Prevalence	Association		
		% (95% CI)	OR (95% CI) crude	OR (95% CI) Adjusted for age and/or sex	OR (95% CI) Adjusted for age and/or sex and vaccination status
Region	Flanders	93.5 (91.6-95.4)	Ref.	Ref.	Ref.
	Brussels	91.7 (89.0-94.3)	0.76 (0.48-1.22)	0.70 (0.44-1.14)	1.24 (0.68-2.25)
	Wallonia	92.2 (89.7-94.8)	0.82 (0.51-1.32)	0.81 (0.50-1.30)	**2.04 (1.14-3.66)**
Sex	Men	92.6 (90.7-94.6)	Ref.	Ref.	Ref.
	Women	93.2 (91.4-95.0)	1.09 (0.74-1.60)	1.09 (0.74-1.61)	1.49 (0.93-2.38)
Age	18-29	96.4 (93.7-99.2)	Ref.	Ref.	Ref.
	30-49	92.7 (90.2-95.3)	0.47 (0.19-1.16)	0.47 (0.19-1.16)	0.52 (0.23-1.19)
	50-69	92.4 (90.0-94.8)	0.45 (0.20-1.03)	0.45 (0.20-1.03)	**0.28 (0.13-0.57)**
	70+	90.4 (87.4-93.5)	**0.35 (0.14-0.84)**	**0.35 (0.14-0.84)**	**0.14 (0.06-0.31)**
Education level	Secondary	91.1 (88.8-93.5)	Ref.	Ref.	Ref.
	Bachelor	93.5 (90.9-96.2)	1.40 (0.84-2.33)	1.29 (0.77-2.16)	1.30 (0.76-2.23)
	Master	95.1 (93.2-97.0)	**1.89 (1.14-3.13)**	1.68 (0.97-2.89)	1.50 (0.81-2.80)

Region

There is no significant association between antibodies seroprevalence and the region of residence, even after adjustment for age and sex. However, when adjusting for vaccination status we find that the antibody seroprevalence is significantly higher in Wallonia than in Flanders (OR_{Adj} 2.04 (95% CI 1.14-3.66)). This can be explained by the higher number of people in Wallonia that reported a history of a COVID-19 infection.

Gender

No association were observed in the prevalence of anti-SARS-CoV-2 antibodies between men and women and this remains the case after adjusting for age and vaccination status.

Age

The prevalence of anti-SARS-CoV-2 antibodies seems to decrease by age category with a statistically significant lower seroprevalence in people aged 70 years and above compared to people aged 18 to 29 years (OR 0.35 (95% CI 0.14-0.84)). This association remains unaltered after adjustment for sex. However, after additional adjustment for vaccination status, the association becomes stronger and a clear age gradient appears indicating a decreasing antibody seroprevalence trend with an increasing age group. This is shown

with an OR_{Adj} of 0.28 (95% CI 0.13-0.57) for the 50-69 age group, and an OR_{Adj} of 0.14 (95% CI 0.06-0.31) for those aged 70 years and older.

Education level

Crude results seem to show a significantly higher seroprevalence in the highest educated people, but after adjustment for age and sex, this association is no longer significant. This is most likely because age is a confounder in the association between the prevalence of anti-SARS-CoV-2 antibodies and educational attainment. Hence there is no evidence for an association between educational level and antibody seroprevalence.

5. SEROCONVERSION / SEROREVERSION BETWEEN WAVE 1 AND WAVE 2

A total of 1486 participants had valid antibody results for wave 1 and wave 2. In Table 6, the results from both waves are combined to form 4 possible categories of seroprevalence evolution. In this report, seroconversion refers to people who changed from a seronegative to a seropositive antibody status between wave 1 and wave 2, whereas seroreversion refers to those who changed from seropositive to seronegative between both waves. Among people with a negative test in wave 1 (n = 578), 87.8% (95% CI 84.9%-90.7%) seroconverted or became positive in wave 2. Among people with a positive test in wave 1 (n = 908), 3.1% (95% CI 1.9%-4.3%) seroreverted or became negative in wave 2.

Table 6 | Anti-SARS-CoV-2 test results in wave 2 in relation to results of wave 1, SalivaHIS study, Belgium 2022

	Number	% (95% CI)
Positive test result in wave 2 among those with positive test result in wave 1	876/908	96.9 (95.7-98.1)
Negative test result in wave 2 among those with positive test result in wave 1	32/908	3.1 (1.9-4.3)
Positive test result in wave 2 among those with negative test result in wave 1	490/578	87.8 (84.9-90.7)
Negative test result in wave 2 among those with negative test result in wave 1	88/578	12.2 (9.3-15.1)

It is informative to link these results with the vaccination status of individuals. E.g. 94.8% (95% CI 92.6%-96.9%) of people who seroconverted from negative to positive received a COVID-19 vaccine between their participation in wave 1 and wave 2. Among the unvaccinated people, only 9.7% (95% CI 0.4%-19.0%) of those with a negative test in wave 1 (n = 44) became positive in wave 2.

It should also be noted that a small percentage (3.1%) of the people who had anti-SARS-CoV-2 antibodies in wave 1 no longer carried antibodies in wave 2. Table 7 provides further information on this subpopulation in function of their vaccination status. Although some numbers are low, it is clear that seroreversion from positive to negative is much more common among unvaccinated people. As regards to the other groups. After adjustment for age and sex, the odds of having seroreverted from positive to negative is ten times higher (OR_{Adj} 10.1 (95% 2.9-35.2) in the unvaccinated or partially vaccinated people as compared to fully vaccinated people. Other analyses were conducted to explore the determinants potentially associated with this seroreversion (e.g. age, sex, presence of chronic disease…), but the sample size was too small to draw valid conclusions on this basis.

Table 7| Percentage of people (18 years or older) with anti-SARS-CoV-2 antibodies in wave 1 who became negative in wave 2, SalivaHIS study, Belgium 2022

Vaccination status in wave 2	Number negative in wave 2 / number positive in wave 1	% seroreversion (positive to negative) (95% CI)
Fully vaccinated > 2w and < 3m	2/142	1.6 (0.0-3.8)
Fully vaccinated > 3 m	25/705	3.0 (1.7-4.3)
Unvaccinated	5/16	24.6 (2.5-46.7)
Total study population	32/908	3.1 (1.9-4.3)

6. TRENDS OF PREVALENCE OF ANTI-SARS-COV-2 ANTIBODIES

6.1. Trends in Belgium and by region.

Table 8 and Figure 2 show the trends in the prevalence of SARS-CoV-2 antibodies in the Belgian population and by region. Results are shown for five periods during which a great enough amount of saliva samples was collected to ensure sufficiently precise population estimates. The first period (29/03-11/04) covers two weeks during which the saliva samples were taken, and this corresponds with the pilot phase of the study. The next saliva collection periods each cover four weeks.

The results show a clear seroprevalence increase in every region, which is in line with the enrollment of the vaccination campaign during the study period (from the end of April 2021 to December 2021), including both the full basic vaccination and from October 2021 onwards, the booster shot.

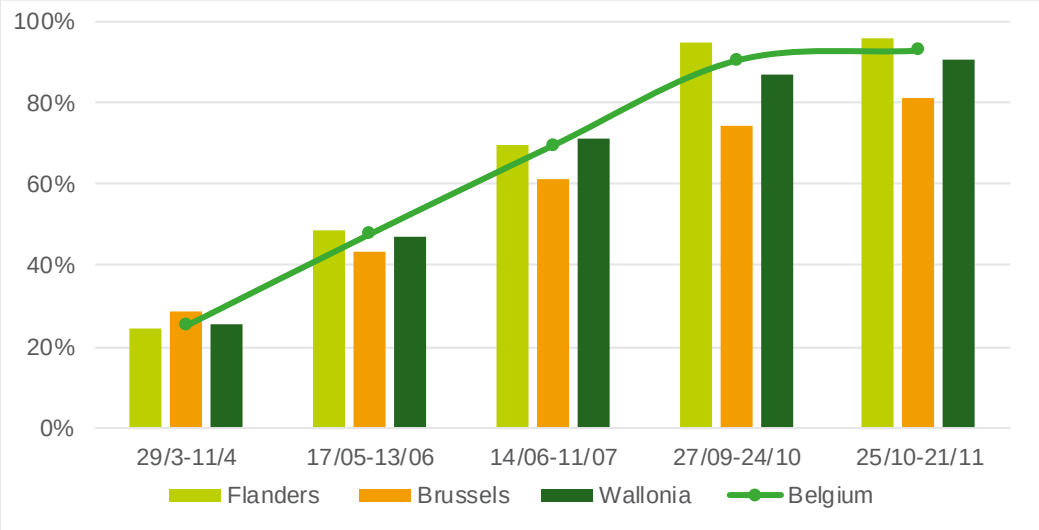
During period 4 (27/09-24/10/2021) the seroprevalence in Flanders reached 95.1% and this percentage only increased slightly (to 96.2%) in period 5 (25/10-21/11/2021). In Brussels and Wallonia, the increase between period 4 and period 5 was bigger (from 61.1% to 74.5% and 71.4% to 86.9% respectively), but the seroprevalence in period 5 still remained lower than in Flanders. Regional differences in seroprevalence can essentially be explained by differences in vaccination rate.

Table 8 | Trends of seroprevalence of anti-Sars-CoV-2 antibodies in the total population (18 years or older), by period of saliva collection, in Belgium and by regions, SalivaHIS study, Belgium 2022

	Belgium		Flanders		Brussels		Wallonia	
Period *2021*	n/N*	Prevalence % (95% CI)	n/N*	Prevalence % (95% CI)	n/N*	Prevalence % (95% CI)	n/N*	Prevalence % (95% CI)
29/3-11/4 $_{w1}$	70/277	25.2 (18.8-31.6)	32/137	24.5 (16.8-32.2)	18/61	28.5 (14.4-42.5)	20/79	25.4 (11.4-39.3)
17/05-13/06 $_{w1}$	595/1089	47.7 (44.0-51.4)	229/445	48.9 (43.6-54.1)	175/301	43.5 (36.3-50.6)	191/343	47.1 (40.6-53.6)
14/06-11/07 $_{w1}$	538/759	69.5 (63.9-75.1)	195/299	69.9 (61.9-77.9)	207/275	61.1 (52.3-69.8)	136/185	71.4 (62.1-80.7)
27/09-24/10 $_{w2}$	758/835	90.4 (87.6-93.2)	341/370	95.1 (92.9-97.1)	192/216	74.5 (61.0-87.9)	225/249	86.9 (81.2-92.7)
25/10-21/11 $_{w2}$	660/710	92.9 (90.2-95.6)	269/286	96.2 (94.7-98.3)	208/226	81.3 (66.8-95.79)	183/198	90.6 (85.3-95.9)

* Number positive/total; $_{w1}$ Wave1; $_{w2}$ Wave 2

Figure 2 | Trends of seroprevalence of SARS-CoV-2 antibodies in the total population (18 years or older), by period of saliva collection in Belgium and by region, SalivaHIS study, Belgium 2022



6.2. Trends of seroprevalence by vaccination status

Table 9 and Figure 3 present the trends of seroprevalence of anti-SARS-CoV-2 antibodies by vaccination status (the vaccination status has been defined in point 2.) using the time periods defined in 6.1. No separate results are presented for the partially vaccinated or having completed the basic vaccination less than 2 weeks ago category because there were only 8 observations in this group in wave 2.

The presentation of the trends by vaccination status poses some problems because the number of fully vaccinated people, and especially the number of fully vaccinated people since more than 3 months is low in wave 1 and the number of unvaccinated people is low in wave 2, which gives some incomplete cells in Table 9. Yet, overall we observe rather stable seroprevalence rates among the vaccinated people. The lower antibody seroprevalence among vaccinated people that is found in period 1 is probably due to the fact that these were mainly older people. Furthermore, there is a tendency that antibody seroprevalence is somewhat higher among the fully vaccinated people who received their last vaccination dose less than 3 months ago than among those who received their last dose longer than 3 months ago (Table 9).

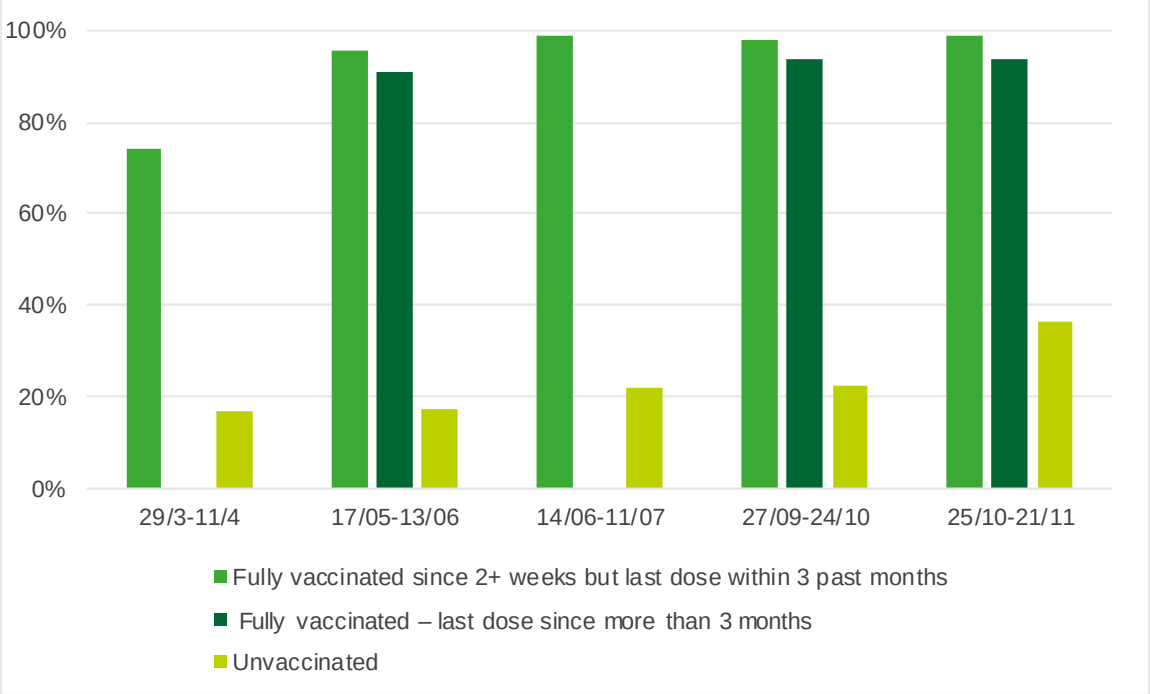
The seroprevalence of anti-SARS-CoV-2 antibodies for the unvaccinated group increased from 16.7% (in week 29/03-11/04) to 36.5% (in week 25/10-21/11), with the most important increase between period 4 and period 5. However, due to the low numbers of unvaccinated people in wave 2, this increase is not statistically significant.

Table 9 | Trends of seroprevalence of anti-SARS-CoV-2 antibodies in the total population (18 years or older), by period of saliva collection and vaccination status, SalivaHIS study, Belgium 2022

	Fully vaccinated >2w and <3 m		Fully vaccinated > 3 months		Unvaccinated	
Period *2021*	n/N*	Prevalence % (95% CI)	n/N*	Prevalence % (95% CI)	n/N*	Prevalence % (95% CI)
29/03-11/04 $_{W1}$	9/12	74.2% (52.2-96.1)	0/0	- (-)	38/223	16.7% (10.1-23.3)
17/05-13/06 $_{W1}$	188/198	95.7% (92.5-98.9)	24/26	91.0% (79.7-100.0)	68/406	17.2% (13.1-21.3)
14/06-11/07 $_{W1}$	261/263	98.9% (97.2-100.0)	37/37	100.0% (-)	34/174	21.7% (10.3-33.3)
27/09-24/10 $_{W2}$	200/208	97.9% (96.3-99.6)	522/563	93.8% (91.6-95.9)	7/33	22.5% (4.9-40.1)
25/10-21/11 $_{W2}$	119/123	99.1% (97.8-100.0)	504/531	95.7% (93.8-97.6)	9/26	36.5% (13.5-59.6)

* Number positive/total $_{W1}$ wave1 $_{W2}$ wave 2

Figure 3 | Trends of seroprevalence of anti-SARS-CoV-2 antibodies in the total population (18 years and older), by period of saliva collection and vaccination status, SalivaHIS study, Belgium 2022



7. FACTORS ASSOCIATED WITH NOT HAVING ANTI-SARS-COV-2 ANTIBODIES

7.1. Potential factors associated with seronegativity

The aim of this section is to identify the potential factors associated with the absence of SARS-CoV-2 antibodies (seronegativity). Since over 90% of the study population had developed antibodies, we were interested in exploring the reasons why some people do not carry antibodies. This is examined separately among the total study population and the vaccinated population.

Logistic regression was used to test the association between SARS-CoV-2 seronegativity and socio-demographic, health related characteristics and COVID-19 related variables. This was done in two steps. First, the association of each independent variable with SARS-CoV-2 seronegativity was assessed by univariate logistic regression. Second, variables found to be significantly associated with seronegativity in the univariate analyses were modeled in a multivariate logistic regression. Associations were quantified using Odds Ratio (ORs) with 95% confidence intervals (95% CI). For all these analyses, a significance level of 5% was used (p-value <0.05). Since the prevalence of not having antibodies is rather rare, occurring in less than 10% of the population, the OR values are good estimates of the relative risks (RR) and associations can be interpreted in this way.

7.1.1. Factors associated with SARS-CoV-2 seronegativity among the total population

Among the study population (Table 10), we assessed the potential association of SARS-CoV-2 seronegativity with socio-demographic variables (i.e. gender, age, region and education), health status related variables (i.e. presence of chronic diseases, body mass index (BMI), blood type, rhesus factor and smoker's status) and COVID-19 related variables (i.e. history of SARS-CoV-2 infection and vaccination status against COVID-19).

The results of the univariate analyses show that the odds of being seronegative (not having anti-SARS-CoV-2 antibodies) were significantly lower among people with a master degree (OR 0.53 (95%CI 0.32-0.88)) compared to those with at most a secondary diploma. On the opposite, the odds of being seronegative were significantly higher among people with overweight (OR 1.88 (95%CI 1.18-3.01)) compared to people with normal weight or underweight. Regarding COVID-19 related variables, having been infected at least once with

the SARS-CoV-2 virus and having been vaccinated against SARS-CoV-2 are negatively associated with the absence of antibodies. This means that the risk of being seronegative is reduced by 77% among people who have had a previous SARS-CoV-2 infection (OR 0.23 (95%CI 0.09-0.55)). Regarding the vaccination status, the risk of being seronegative is reduced by 99% for people fully vaccinated since less than 3 months (OR 0.01 (95%CI 0.00-0.02)), 97% for people fully vaccinated since more than 3 months (OR 0.03 (95%CI 0.01-0.05)) and 85% for people partially vaccinated (OR 0.15 (95%CI 0.03-0.95)) compared to unvaccinated people.

All variables found to be significantly associated with seronegativity in the univariate analyses were modelled in a multivariate model. The results show that, taking into account confounding, the odds of not having anti-SARS-CoV-2 antibodies versus having antibodies were significantly higher in people of 50 years and older (OR 2.26 (95%CI 1.28-3.98)) than younger people (18-49 years and above). After adjustment for the other variables, the risk of not having antibodies is reduced by 92% among people who have had a previous SARS-CoV-2 infection (OR 0.08 (95%CI 0.02-0.31)). Furthermore, the risk of not having antibodies is reduced by 99.7% for people fully vaccinated for less than 3 months (OR 0.003 (95%CI 0.00-0.01)), 99% for people fully vaccinated for more than 3 months (OR 0.01 (95%CI 0.00-0.03)) and 84% for people partially vaccinated (OR 0.16 (95%CI 0.03-0.81)) compared to unvaccinated people.

7.1.2. Factors associated with SARS-CoV-2 seronegativity among fully vaccinated population

In a next step, potential determinants of seronegativity among the vaccinated population were assessed via univariate and multivariate analyses (Table 11). We assessed the potential association of SARS-CoV-2 seronegativity with socio-demographic variables (i.e. gender, age, region and education), health status related variables (i.e. presence of chronic diseases, BMI and smoker's status) and COVID-19 related variables (i.e. type of vaccine received and number of days since the last vaccination).

Results show, in the univariate model, that the odds of not having anti-SARS-CoV-2 antibodies were significantly higher in people of 50 years and older (OR 3.34 (95% CI 1.87-5.99)) compared to younger people, in people with at least one chronic disease (OR 1.88 (95% CI (1.10-3.20)) compared to those having no chronic disease, and in people with overweight (OR 2.38 (95% CI 1.35-4.19)) compared to normal or underweight people. After adjustment for the other variables, age and the presence of at least one chronic disease remained significantly associated with the absence of anti-SARS-CoV-2 antibodies. In the multivariate analysis, the risk of being seronegative is reduced by 50% in Wallonia (OR 0.50 (95% CI 0.26-0.95)) compared to Flanders. This association could be explained by a higher number of infections in Wallonia than in Flanders during the study period. The presence of SARS-CoV-2 infection was not considered in this model.

Regarding COVID-19 related variables, the risk of being seronegative is reduced by 86% among people who received at least one dose of mRNA vaccine (OR 0.14 (95% CI (0.08-0.23)) compared to those who received adenoviral-vectored vaccine in the univariate model. This association remained significant in the multivariate model (OR 0.10 (95% CI 0.05-0.19)). This result is in the line with the ones highlighted in wave 1. Furthermore, an association with the number of days since the last vaccination is also highlighted. Indeed, the risk of being seronegative significantly increases with an increase in the number of days since the last vaccination (OR 1.01 (95% CI 1.00-1.02)).

Table 10 | Prevalence of the absence of anti-SARS-CoV-2 antibodies and potential factors associated among total population aged 18 years or older using univariate and multivariate logistics regression, SalivaHIS study, wave 2, Belgium 2022

Determinants	Categories	Total population (N=1698) Prevalence		Association	
		%	Total N	Unadjusted OR (95% CI)	Adjusted OR_{Adj} (95% CI)
Gender	Man	7.4	70	Ref.	Ref.
	Woman	6.8	67	0.92 (0.62-1.35)	0.78 (0.47-1.29)
Age	18-49 yrs	6.0	48	Ref.	Ref.
	≥50 yrs	8.2	89	1.40 (0.94-2.10)	**2.26 (1.28-3.98)°**
Region	Flanders	6.5	51	Ref.	Ref.
	Brussels	8.3	46	1.31 (0.82-2.10)	1.15 (0.62-2.13)
	Wallonia	7.8	10	1.22 (0.76-1.96)	0.58 (0.32-1.04)
Education	≤Secondary	8.9	60	Ref.	Ref.
	Bachelor	6.5	32	0.71 (0.43-1.19)	0.68 (0.36-1.32)
	≥Master	4.9	39	**0.53 (0.32-0.88)°**	0.68 (0.39-1.20)
Presence of at least one chronic disease	Yes	8.5	39	1.31 (0.81-2.12)	
	No	6.6	89	Ref.	
BMI	<25kg/m²	5.2	52	Ref.	Ref.
	25-30kg/m²	9.3	52	**1.88 (1.18-3.01)°**	1.65 (0.91-2.97)
	≥30kg/m²	7.8	26	1.55 (0.88-2.70)	1.28 (0.66-2.46)
Blood type	O blood type	6.7	47	Ref.	
	Non O blood type	7.3	41	1.10 (0.68-1.79)	
Rhesus type	Positive	6.9	69	0.94 (0.48-1.84)	
	Negative	7.4	15	Ref.	
Smokers	Yes	10.1	23	1.59 (0.92-2.75)	
	No	6.6	108	Ref.	
SARS-CoV-2 infection	At least one infection	1.9	7	**0.23 (0.09-0.55)***	**0.08 (0.02-0.31)***
	Not infected	7.7	127	Ref.	Ref.
Vaccination status	Fully vaccinated since >3 months	5.5	73	**0.03 (0.01-0.05)***	**0.01 (0.00-0.03)***
	Fully vaccinated since <3 months	2.0	12	**0.01 (0.00-0.02)***	**0.003 (0.00-0.01)***
	Partially vaccinated	26.0	2	**0.15 (0.03-0.95)°**	**0.16 (0.03-0.81)°**
	Not vaccinated	69.6	46	Ref	Ref

*°p-value <0.05; * p-value<0.001*

Table 11 | Prevalence of seronegativity and potential factors associated among vaccinated population (18 years or older) using univariate and multivariate logistic regression, SalivaHIS study, wave 2, Belgium 2022

Determinants	Categories	Fully vaccinated population (N=1557)			
		Prevalence		Association	
		%	Total N	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Gender	Man	5.5	53	Ref	Ref
	Woman	3.8	32	0.68 (0.41-1.13)	0.60 (0.34-1.08)
Age	18-49 yrs	2.3	17	Ref	Ref
	≥50 yrs	7.2	68	**3.34 (1.87-5.99)***	**2.05 (1.07-3.93)°**
Region	Flanders	5.3	42	Ref	Ref
	Brussels	4.6	24	0.86 (0.49-1.53)	1.00 (0.53-1.87)
	Wallonia	3.4	19	0.64 (0.35-1.15)	**0.50 (0.26-0.95)°**
Education	≤Secondary	6.2	38	Ref	
	Bachelor	3.4	17	0.54 (0.28-1.04)	
	≥Master	3.7	27	0.59 (0.32-1.08)	
Presence of at least one chronic disease	Yes	7.0	30	**1.88 (1.10-3.20)°**	**1.92 (1.07-3.46)°**
	No	3.9	49	Ref	Ref
BMI	<25kg/m²	3.0	29	Ref	Ref
	25-30 kg/m²	6.9	36	**2.38 (1.35-4.19)°**	1.74 (0.91-3.33)
	≥30kg/m²	4.5	16	1.53 (0.75-3.15)	0.90 (0.42-1.97)
Smokers	Yes	6.7	13	1.58 (0.79-3.15)	
	No	4.4	70	Ref	
Type of vaccination	At least one done of mRNA	2.2	31	**0.14 (0.08-0.23)***	**0.10 (0.05-0.19)***
	Adenoviral-vectored	14.2	54	Ref	Ref
Number of days since the last vaccination				1.00 (1.00-1.01)	**1.01 (1.00-1.02)°**

°***p-value <0.05; * p-value <0.001***

8. REFERENCES

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