Prevalence of Anti-SARS-CoV-2 Antibodies and Potential Determinants among the Belgian Adult Population: Baseline Results of a Prospective Cohort Study

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Abstract: The prevalence of anti-SARS-CoV-2 antibodies and potential determinants were assessed in a random sample representative of the Belgian adult population. In total, 14,201 individuals (≥18 years) were invited by mail to provide saliva via an Oracol® swab. Survey weights were applied, and potential determinants were estimated using multivariable logistic regressions. Between March and August 2021, 2767 individuals participated in the first data collection. During this period, which coincided with the onset of the vaccination campaign, the seroprevalence in the population increased from 25.2% in March/April to 78.1% in July. Among the vaccinated there was an increase from 74.2% to 98.8%; among the unvaccinated, the seroprevalence remained stable (around 17%). Among the vaccinated, factors significantly associated with the presence of antibodies were: having at least one chronic disease (OR 0.22 (95% CI 0.08–0.62)), having received an mRNA-type vaccine (OR 5.38 (95% CI 1.72–16.80)), and having received an influenza vaccine in 2020–2021 (OR 3.79 (95% CI 1.30–11.07)). Among the unvaccinated, having a non-O blood type (OR 2.00 (95% CI 1.09–3.67)) and having one or more positive COVID-19 tests (OR 11.04 (95% CI 4.69–26.02)) were significantly associated. This study provides a better understanding of vaccine-and/or natural-induced presence of anti-SARS-CoV-2 antibodies and factors that are associated with this presence.

Keywords: SARS-CoV-2; COVID-19; antibodies; seroprevalence; cohort; population-based study; Belgium

1. Introduction

On 11 March 2020, the World Health Organization (WHO) declared the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) outbreak as a pandemic [1]. In Belgium, the first confirmed SARS-CoV-2 case was reported on 2 February 2020 [2]. From then, the virus spread rapidly, causing a high number of infections, hospitalizations, and casualties [3].

Since the beginning of the pandemic, large efforts have been directed towards finding a treatment and vaccine. The first vaccines against SARS-CoV-2 became available in December 2020 and in January 2021, the Belgian vaccination campaign was launched, starting with the vaccination of priority groups as defined by the Belgian Superior Health Council, being health care workers, people aged 65 years and above, and people younger than 65 years at risk of developing a severe form of COVID-19 due to existing comorbidities [4].

Epidemiological surveillance to monitor the COVID-19 epidemic were established. This included a systematic collection, analysis, and interpretation of the number of COVID-19 cases, hospitalizations, ICU patients, deaths, and a follow up of the vaccination rate [5,6].
Additionally, the knowledge on the prevalence of anti-SARS-CoV-2 antibodies in the population is important to better understand the epidemiological situation and provide, among others, valuable information regarding vaccine-induced and/or natural immunity against SARS-CoV-2 infections [7]. Furthermore, this enhances the understanding of the antibody response following vaccination by identifying factors that may explain the presence or absence of antibodies, the duration of the presence of these antibodies, or the protection against other infections. Monitoring the presence of antibodies among unvaccinated people further allows a better estimation of the virus circulation by identifying, for example, infected but asymptomatic subjects, potentially missed by the usual test and tracing system.

COVID-19 seroprevalence studies have been conducted in several countries; as, among others, in Switzerland [8], Portugal [9], Austria [10], and England [11]. In Belgium, studies have first been set up in specific population groups such as blood donors [12], hospital healthcare workers [13], primary healthcare workers [14], schoolchildren and school staff [15], and residents and staff in nursing homes [16]. The SalivaHIS study is complementary to these studies as it assesses the prevalence of anti-SARS-2-CoV antibodies in the community dwelling population aged 18 years and older. In the SalivaHIS study, the presence of anti-SARS-CoV-2 antibodies is measured in saliva.

Seroprevalence studies are mostly performed using serologic assays detecting anti-SARS-CoV-2 antibodies in serum or blood [17]. These methods are the reference to assess if a person had antibodies against COVID-19 or not. However, serological-based studies may be more difficult to implement in the general population because of logistical and practical constraints to obtain a serum sample in a geographically scattered random population. To overcome this issue, we considered the detection of antibodies in the saliva as an acceptable non-invasive alternative to serological testing to monitor SARS-CoV-2 antibody development [18,19]. Experiences in the US and Belgium showed that the use of saliva-based antibody testing is a scalable alternative to blood-based antibody testing [18,20].

This study aims to assess the prevalence of anti-SARS-CoV-2 antibodies among the general adult population in Belgium during the period March–August 2021 and to investigate to what extent this prevalence varies in function of socio-demographic characteristics, health status, and COVID-19-related health behavior, both among the vaccinated and unvaccinated populations.

2. Materials and Methods

We followed for this paper the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) [21] recommendations. The study research protocol is available on the Open Science Framework platform: https://osf.io/5tf8s/ (accessed on 27 April 2022).

2.1. Design, Setting, and Study Population

The SalivaHIS study is a population-based prospective cohort study in which a saliva sample and questionnaire data are self-collected from a sample of the Belgian adult population.

This paper reports the results of the first testing period which took place between 25 March and 31 August 2021. Additional testing periods are planned after 3 and 6 months. Findings reported in this paper do not include data from the follow-up testing periods; hence, this study has a cross-sectional design.

The target population consists of people aged 18 years and above residing in private dwellings in Belgium without any restrictions and regardless of their use of health services. The sample frame is the National Register, which includes all citizens with an official address in Belgium. The primary selection unit was the household and all residents aged 18 years and older of the selected households were eligible for participation. Households were selected through a stratified random sampling method using 24 strata defined by the region of residence (Flanders, Brussels, capital, and Wallonia), sex, and age group of the reference person of the household, as defined in the National Register.
The fieldwork had a pilot phase (from 29 March to 5 May 2021) including 1339 individuals belonging to 634 households and the main study phase (from 17 May to 28 August 2021) including 12,862 individuals belonging to 7598 households. Strata information on the participation rate by stratum in the pilot phase was used to decide on the number of households to be invited per stratum for the main study. As the study procedures did not change between the pilot phase and the main study, the participants of the pilot phase were integrated into the main study sample.

2.2. Data Collection

People selected for the study received an invitation letter by regular mail, two consent forms, an Oracol® tube (Malvern Medical Developments Ltd., Worcester, Worcestershire, UK) to collect saliva, an instruction document on how to collect the saliva and how to obtain test results, a paper questionnaire, and a prepaid return envelope. An identification code was assigned to all participants to create pseudonymity and to link the different data collection materials. For privacy reasons, researchers had no access to any personal information of the respondents and invitations were sent through a trusted third party, Statbel, the Belgian national office of statistics. To participate in the study, it was required to send back a saliva sample, a consent form, and a completed questionnaire. It was also possible to complete the questionnaire online. The topics in the questionnaire selected based on their relevance for the analysis of determinants of COVID-19 seroprevalence status were: (1) sociodemographic information, (2) presence of chronic diseases, (3) occupational status, (4) financial situation, (5) access to health care services, (6) mental health, (7) social contacts, (8) lifestyle, (9) possible contact with SARS-CoV-2 virus and consequences, (10) adherence to corona measures, (11) vaccination status, and (12) attitude towards vaccination.

Invitations for the pilot phase were sent on 25 March 2021, invitations for the main study between 18 May and 15 June 2021 in three batches. The spread of the mailing over a period of 4 weeks was done to be able to assess the evolution of the outcome indicators across the study period.

2.3. Outcomes and Potential Determinants

The main outcome was the presence of anti-SARS-CoV-2 antibodies among the general population. The saliva samples were analyzed by the laboratories in Sciensano. The detection of anti-SARS-CoV-2 antibodies in saliva was completed using the WANTAI SARS-CoV-2 IgG ELISA (Wantai Bio-Pharm, cat n° WS-1396), a semi-quantitative measure of anti-RBD (Receptor Binding Domain) IgG, customized for saliva (in house protocol). The cut-off value for anti-RBD IgG positivity in saliva was established using well-characterized PCR-confirmed samples from adults of which corresponding serum and saliva were available. Using the predefined positivity cut off (cut off value > 2), a binary result was provided by the laboratory, classifying each sample as positive versus negative for the presence of antibodies against SARS-CoV-2. This cut-off resulted in a specificity of 98.9% and a sensitivity of 91.8%.

Vaccination status was assessed using questionnaire data. At the time of the study period, four vaccine types were administered in Belgium: AstraZeneca/ChAdOx1-S, Pfizer/BNT1272b2, Moderna/mRNA-1273, and Janssen/Ad26.COV2.S. People were considered as fully vaccinated if they had received, since at least two weeks, one dose of the Janssen/Ad26.COV2.S vaccine or two doses of the other vaccines. The questionnaire data also allowed to identify people who were aware of having had a close contact with a COVID-19 positive person and those who had one or more positive COVID-19 test results themselves.

Determinants that were hypothesized to potentially affect the presence of anti-SARS-CoV-2 antibodies included demographic characteristics (age, gender, region of residence), living situation, household size, education level, and occupation status. Health-related and biological factors which may also be relevant for infection with the SARS-CoV-2 virus were addressed.
Overweight was defined as a body mass index (BMI) ≥ 25 kg/m² and obesity as a BMI ≥ 30 kg/m². Chronic diseases that were listed included diseases to which the Belgian Superior Health Council allocated a priority status regarding vaccination, being: chronic lung disease, chronic cardiovascular disease, diabetes, chronic neurological disease, cancer (including blood cancer), chronic renal disease, immune compromised (except HIV), and transplant patients. An indicator was created identifying people with at least one chronic disease and one with at least two chronic diseases.

Health-related behavior considered in this study included smoking status and adherence to public health preventive measures implemented in the framework of the corona crisis. Nine common COVID-19 measures were taken into consideration. People who indicated having strictly complied with all nine measures were categorized as having “strictly followed them”; people who reported they strictly complied with 5–8 out of the 9 measures were categorized as having “moderately followed them”; and those who complied with less than 5 measures were referred as having “insufficiently followed the measures”.

2.4. Sample Size

The main outcome indicator of the study was the prevalence of anti-SARS-CoV-2 antibodies among the general population. Sample size calculations indicated that a sample size of 1200 individuals in each of the 3 Belgian regions would be large enough to obtain regional estimates on the main outcome indicator with a sufficiently high precision. More specifically, assuming a prevalence of anti-SARS-CoV-2 antibodies in the population of 50% and taking into account an alpha error of 0.05, these sample sizes would yield a margin of error of 1.9% at the level of Belgium and 3.3% at the level of the regions.

2.5. Time Periods Considered for Trend Analyses

Saliva samples were returned to the lab between 29 March (week 13) and 27 August 2021 (week 34). Figure 1 shows that the collection of saliva samples was not equally spread over this study period. Too few saliva samples were collected in the weeks between April 12 and May 16, corresponding to the period between the pilot phase and the launch of the main study, and after July 11 to have sufficiently precise estimates for those time periods. It was therefore decided to focus for the trend analysis on five periods of two weeks for which at least 200 saliva samples were collected (Table 1).

Figure 1. Number of saliva samples collected in the SalivaHIS study per week.
Table 1. Time periods considered for trend analyses.

| Periods | Week of References | Dates                  |
|---------|--------------------|------------------------|
| Period 1| Week 13–14         | 29 March to 11 April 2021 |
| Period 2| Week 20–21         | 17 May to 30 May 2021  |
| Period 3| Week 22–23         | 31 May to 21 June 2021 |
| Period 4| Week 24–25         | 14 June to 27 June 2021 |
| Period 5| Week 26–27         | 28 June to 11 July 2021 |

2.6. Statistical Analyses

Initial weighted seroprevalence estimates were calculated with 95% confidence intervals, taking into account the design of the survey. Post stratification survey weights were calculated based on the population structure on the 1 January 2021 obtained from Statbel as the auxiliary reference database. The effect of the household cluster was also taken into account in the survey design settings.

Correlates of the presence of anti-SARS-CoV-2 antibodies were assessed through logistic regression analysis. Because associations between having anti-SARS-CoV-2 antibodies and other potential determinants may differ for the vaccinated and the unvaccinated populations, the choice of variables to be included in the models was not the same in both groups. In the model exploring determinants of seroprevalence among the vaccinated population, variables that could influence the occurrence of antibodies in this particular population were explored (e.g., type of vaccine, presence of chronic disease, etc.). The presence of antibodies among the unvaccinated population is directly linked to the exposure to the virus. In the model exploring determinants of seroprevalence in this population, other variables were explored, such as history of a COVID-19 infection, contact with a positive person or compliance with preventive measures against the spread of the virus.

To assess time trends during the study period, data collections were analyzed as repeated cross-sectional data. Post stratification survey weights were calculated for each two-week period separately. To take into account a differential participation rate between vaccinated and unvaccinated people, weights to assess the trends in the prevalence of anti-SARS-CoV-2 antibodies took into account regional, age, and gender differences between the sample and the general population, but also the differences in the vaccination status at each of the given periods. This was completed by multiplying the initial total sample weight with a correction factor. The correction factor was obtained by dividing the number of people by region and vaccination status in the population by the number of participants by region and vaccination status in the sample. This way, the sample distribution weighted by region and vaccination status matched this distribution in the general population.

Logistic regression analysis was applied to assess time trends between the 5 time-periods. The longer time lag between the first two-week period (week 13–14) and the four other periods (week 20–27) was taken into account in the analysis.

3. Results

3.1. Description of the Population

Figure 2 gives an overview of the number of individuals invited, the participation rate and the net sample obtained in the pilot phase and in the main study for the three Belgian Regions. A total of 14,201 individuals were invited to participate in the SalivaHIS study. At the first testing period, the study included 2767 participants (with a participation rate of 19.5%) of which 2288 (82.6%) had a saliva sample with sufficient salivary volume to ensure high quality analysis.
An overview of the characteristics of the study population and their vaccination status is presented in Table 2. In summary, 54.9% of the participants were women, 46.4% were between 18 and 49 years old, 42.7% had at most a high school diploma, and 8.1% were a healthcare worker. As a result of the Belgian vaccination strategy at the time of the study, which focused on priority groups, vaccinated people were relatively older and included a higher share of women, health care workers, people with at least one chronic disease, and people vaccinated against influenza. As compared to the vaccinated, the unvaccinated were younger, more often men and smokers, as well as people who reported to have tested positive for COVID-19.

Table 2. Description of the study participants.

| Overall Distribution | Fully Vaccinated Population Since 2 or More Weeks N = 747 (28.5%) | Partially Vaccinated Population N = 874 (33.4%) | Unvaccinated Population N = 998 (38.1%) |
|----------------------|---------------------------------------------------------------|-------------------------------------------------|-------------------------------------|
| Study Population N = 2767 | % Total N | % Total N | % Total N | % Total N |
| Gender | Man | 45.1 1247 | 39.5 295 | 46.5 406 | 46.5 464 |
| | Woman | 54.9 1520 | 60.5 452 | 53.5 468 | 53.5 534 |
| Age | 18–49 yrs | 46.4 1285 | 21.6 161 | 35.6 311 | 75.1 749 |
| | ≥50 yrs | 53.6 1482 | 78.4 586 | 64.4 563 | 24.9 249 |
| Region | Flanders | 41.9 1160 | 34.1 255 | 42.8 374 | 47.6 475 |
| | Brussels | 29.6 819 | 36.7 274 | 27.0 236 | 26.1 260 |
| | Wallonia | 28.5 788 | 29.2 218 | 30.2 264 | 26.4 263 |
| Education | ≤Secondary | 42.7 1138 | 40.2 286 | 42.5 359 | 44.7 437 |
| | Bachelor | 27.1 722 | 29.4 209 | 28.8 243 | 24.5 239 |
| | ≥Master | 30.2 807 | 30.5 217 | 28.8 243 | 30.8 301 |
| Household size | 1 member | 15.2 382 | 21.6 153 | 15.4 129 | 10.4 100 |
| | 2 members | 44.8 1124 | 54.0 383 | 49.4 414 | 34.1 327 |
| | 3 members | 15.5 389 | 9.9 70 | 14.8 124 | 20.3 195 |
| | ≥4 members | 24.4 641 | 14.5 103 | 20.4 171 | 35.2 338 |
| Health care worker | Yes | 8.1 214 | 17.8 128 | 5.2 43 | 3.5 34 |
| | No | 91.9 2423 | 82.2 592 | 94.8 786 | 96.5 924 |
| Presence of at least one chronic disease | Yes | 24.3 643 | 32.8 235 | 25.0 207 | 17.1 165 |
| | No | 75.7 2002 | 67.2 482 | 75.0 622 | 82.9 801 |
| Presence of at least two chronic diseases | Yes | 5.7 151 | 8.1 58 | 6.2 51 | 3.8 37 |
| | No | 94.3 2494 | 91.9 659 | 93.8 778 | 96.2 929 |
**Table 2. Cont.**

| Study Population | Fully Vaccinated Population Since 2 or More Weeks N = 747 (28.5%) | Partially Vaccinated Population N = 874 (33.4%) | Unvaccinated Population N = 998 (38.1%) |
|------------------|---------------------------------------------------------------|-------------------------------------------------|----------------------------------------|
| **Overweight**   |                                                               |                                                 |                                        |
| Yes              | 48.8 1299                                                     | 54.4 392                                        | 51.3 430 41.8 407                      |
| No               | 51.2 1365                                                    | 45.6 329                                        | 48.7 409 58.2 566                      |
| **Obesity**      |                                                               |                                                 |                                        |
| Yes              | 16.6 441                                                     | 21.1 152                                        | 16.7 140 12.9 126                      |
| No               | 83.4 2223                                                    | 78.9 569                                        | 83.3 699 87.1 847                      |
| **Blood type**   |                                                               |                                                 |                                        |
| O blood type     | 56.7 1020                                                    | 58.0 286                                        | 58.1 349 52.8 331                      |
| Non-O blood type | 43.3 780                                                     | 42.0 207                                        | 41.9 252 47.2 296                      |
| **Rhesus**       |                                                               |                                                 |                                        |
| Positive         | 81.8 1411                                                    | 81.4 380                                        | 81.8 472 82.9 503                      |
| Negative         | 18.2 313                                                     | 18.6 87                                         | 18.2 105 17.1 104                      |
| **Smokers**      |                                                               |                                                 |                                        |
| Yes              | 14.7 394                                                     | 10.1 73                                         | 13.4 114 19.5 191                      |
| No               | 85.3 2280                                                    | 89.9 647                                        | 86.6 735 80.5 786                      |
| **Influenza vaccine** |                                               |                                                 |                                        |
| Vaccinated       | 37.4 1011                                                    | 62.7 458                                        | 42.2 360 14.4 142                      |
| Not vaccinated   | 62.6 1693                                                    | 37.3 273                                        | 57.8 493 85.6 844                      |
| **Close contact with COVID-19 positive person** | | | |
| Yes              | 27.2 737                                                     | 23.3 170                                        | 22.7 194 34.1 337                      |
| No               |                                                               |                                                 |                                        |
| I do not know    | 56.3 1523                                                    | 61.5 449                                        | 61.7 528 47.6 470                      |
| **One or more positive COVID-19 test results** | | | |
| Yes              | 11.8 306                                                     | 11.0 78                                         | 10.7 87 12.8 121                      |
| No               |                                                               |                                                 |                                        |
| I do not know    | 88.2 2289                                                    | 89.0 633                                        | 89.3 723 87.2 822                      |
| **Preventive measures** |                                               |                                                 |                                        |
| Strictly followed the measures | 30.3 555                                                | 38.9 189                                        | 33.6 192 21.3 148                      |
| Moderately followed the measures | 45.4 831                                                | 47.1 229                                        | 46.0 263 44.2 307                      |
| Insufficiently followed the measures | 24.3 444                                                | 14.0 68                                         | 20.5 117 34.4 239                      |

3.2. Prevalence of Anti-SARS-CoV-2 Antibodies over Time among the Study Population and by Vaccination Status

Figure 3 presents the prevalence of anti-SARS-CoV-2 antibodies among the total study population and by vaccination status, by two-week periods of saliva reception as a cross-sectional collection. Among the total study population, the prevalence of anti-SARS-CoV-2 antibodies increased significantly from 25.2% (95% CI: 18.8–31.6) in the first period (week 13–14) to 78.1% (95% CI: 69.2–87.0) in the last period studied (week 26–27). Among the unvaccinated, a not significant increase in the prevalence of anti-SARS-CoV-2 antibodies was observed between the fourth study period (week 24–25: 16.8% 95% CI: 9.8–23.7) and the last study period (week 26–27: 28.9% 95% CI: 4.0–53.7). However, caution is needed to interpret this increase, because the number of saliva samples for the last study period was rather low. Among the vaccinated, from the second period onwards, the prevalence of anti-SARS-CoV-2 antibodies was above 90% and stable (ranging between 93.1% (95% CI 88.3–97.8) and 97.9% (95% CI 93.9–100)). At the first period, this prevalence was remarkably lower (week 13–14: 74.2% (95% CI 52.2–96.1)).
3.3. Determinants of the Prevalence of Antibodies among Vaccinated and Unvaccinated Populations

Table 3 shows the prevalence of anti-SARS-CoV-2 antibodies and the results of the logistic regression investigating determinants with having antibodies for both vaccinated and unvaccinated populations.

### Table 3. Prevalence of anti-SARS-CoV-2 antibodies among vaccinated and unvaccinated populations and potential factors associated.

| Determinants | Categories | Vaccinated Population (N = 593) | Unvaccinated Population (N = 838) |
|--------------|------------|---------------------------------|-----------------------------------|
|              |            | % | Total N | Unadjusted OR (95% CI) | Adjusted $^*$ OR (95% CI) | % | Total N | Unadjusted OR (95% CI) | Adjusted $^*$ OR (95% CI) |
| Gender       | Man        | 94.2 | 250 | Ref | Ref | 15.3 | 398 | Ref | Ref |
|              | Woman      | 95.6 | 343 | 1.32 | 0.57–3.12 | 1.09 | 0.45–2.68 | 21.0 | 440 | 1.46 | 1.01–2.12 | 1.33 | 0.72–2.49 |
| Age          | 18–49 yrs  | 97.4 | 137 | Ref | Ref | 18.5 | 638 | Ref | Ref |
|              | ≥50 yrs    | 94.1 | 456 | 0.43 | 0.13–1.39 | 0.34 | 0.08–1.45 | 16.1 | 200 | 0.96 | 0.72–1.28 | 0.87 | 0.45–1.67 |
| Region       | Flanders   | 97.7 | 196 | Ref | Ref | 16.4 | 388 | Ref | Ref |
|              | Brussels   | 97.8 | 228 | 2.27 | 0.78–6.60 | 2.07 | 0.59–7.30 | 22.9 | 225 | 1.52 | 0.97–2.37 | 0.76 | 0.34–1.69 |
|              | Wallonia   | 93.9 | 169 | 0.81 | 0.30–2.15 | 1.25 | 0.40–3.90 | 19.6 | 225 | 1.25 | 0.74–2.10 | 0.84 | 0.34–2.05 |
| Education    | ≤Secondary | 93.8 | 219 | Ref | Ref | 17.2 | 358 | Ref | Ref |
|              | Bachelor   | 95.9 | 169 | 1.54 | 0.51–4.70 | 20.9 | 206 | 1.27 | (0.80–2.02) |
|              | ≥Master    | 96.4 | 177 | 1.78 | 0.50–6.32 | 16.8 | 254 | 0.97 | 0.61–1.56 |
| Household size | 1 member  | 95.6 | 117 | Ref | Ref | 20.0 | 82 | Ref | Ref |
|              | 2 members  | 95.1 | 303 | 0.89 | 0.22–3.54 | 12.5 | 269 | 0.57 | 0.27–1.22 |
|              | 3 members  | 88.2 | 61 | 0.34 | 0.07–1.65 | 13.5 | 174 | 0.62 | 0.27–1.44 |
|              | ≥4 members | 97.9 | 84 | 2.13 | 0.29–15.84 | 25.0 | 282 | 1.33 | 0.64–2.77 |

Figure 3. Prevalence of anti-SARS-CoV-2 antibodies by 2-week period of saliva collection.
Table 3. Cont.

| Determinants | Categories                        | Vaccinated Population (N = 593) | Unvaccinated Population (N = 838) |
|--------------|-----------------------------------|---------------------------------|-----------------------------------|
|              | % Total N Unadjusted OR (95% CI)   | % Total N Unadjusted OR (95% CI) |
|              | Adjusted OR (95% CI)              | Adjusted OR (95% CI)            |
| Health care worker | Yes 95.2 103 0.99 (0.31–3.13) | 17.4 33 0.99 (0.38–2.57) |
|              | No 95.2 470 Ref                   | 17.6 770 Ref                    |
| Presence of at least one chronic disease | Yes 90.4 173 0.30 (0.12–0.76) * | 21.0 125 1.29 (0.78–2.14) |
|              | No 97.0 400 Ref                   | 17.1 684 Ref                    |
| Presence of at least two chronic diseases | Yes 93.9 41 0.81 (0.18–3.73) | 19.7 27 1.15 (0.42–3.11) |
| BMI 25–30 kg/m² | Yes 93.8 306 0.59 (0.23–1.48) | 18.5 335 (0.75–1.66) |
|              | No 96.3 271 Ref                   | 16.8 481 Ref                    |
| BMI > 30 kg/m² | Yes 95.0 108 1.02 (0.36–2.88) | 17.2 103 (0.55–1.72) |
|              | No 94.9 469 Ref                   | 17.6 713 Ref                    |
| Blood type   | O blood type                      | 1.83 22.5 236 (1.11–3.01) *    |
|              | Non-O blood type                  | 1.87 291 Ref                    |
| Rhesus       | Positive                          | 15.6 422 (0.32–1.00)            |
|              | Negative                          | 24.9 90 Ref                     |
| Smokers      | Yes 8.5 161 (0.18–0.72) *        |
|              | No 20.2 658 Ref                   | 0.64 (0.14–1.33)               |
| Close contact with COVID-19 positive person | Yes 29.9 282 3.28 (2.08–5.17) * | 1.44 (0.71–2.92) |
|              | No I do not know                  | 11.5 388 Ref                    |
|              | 14.2 159 Ref                      | 1.27 (0.69–2.31)               |
| One or more positive COVID-19 test results | Yes 68.7 103 17.62 (10.16–30.57) * | 11.04 (4.69–26.02) * |
| Preventive measures | No Strictly followed the measures | 11.1 690 Ref                    |
|              | Moderately followed the measures  | 16.1 118 Ref                    |
|              | Insufficiently followed the measures | 19.4 261 1.26 (0.69–2.31) |
| Influenza vaccine | Vaccinated 96.8 361 2.62 (1.06–6.49) * | 15.5 104 0.85 (0.46–1.57) |
|              | Not vaccinated adenoviral-vectored vaccine 92.0 222 Ref | 0.85 (0.46–1.57) |
| Type of vaccination | Adenoviral vectored 96.5 461 5.10 (1.89–13.79) * | 5.38 (1.72–16.80) * |
|              | mRNA vaccine 84.3 58 Ref          | 17.8 722 Ref                    |
Table 3. Cont.

| Determinants                                      | Categories | Vaccinated Population (N = 593) | Unvaccinated Population (N = 838) |
|--------------------------------------------------|------------|---------------------------------|-----------------------------------|
|                                                  |            | Unadjusted $^a$ OR (95% CI)     | Adjusted $^a$ OR (95% CI)         |
|                                                  |            | % Total N                        | % Total N                         |
| Number of days since the last vaccination        |            | 1.00 (0.99–1.02)                | 11.04 (95% CI 4.69–26.02)         |

$^a$ The association of each independent variable with having SARS-CoV-2 antibodies was assessed individually by univariate logistic regression. $^a$ All variables found to be significantly associated with having anti-SARS-CoV-2 antibodies in the univariate analyses were modeled in a multivariable logistic regression. $^*$ p-value < 0.001.

3.3.1. Vaccinated Population

With the exception of those vaccinated with an adenoviral-vectored vaccine type (i.e., AstraZeneca/ChAdOx1-S and Janssen/Ad26.COV2.S vaccine), the prevalence of anti-SARS-CoV-2 antibodies was over 90% among the vaccinated population. In the univariate analyses, no significant differences in the prevalence of anti-SARS-CoV-2 antibodies were observed by age, sex, region of residence, level of education, household size, being a healthcare worker or not, being overweight or obese, or the number of days since the last COVID-19 vaccination. The odds of having anti-SARS-CoV-2 antibodies was significantly lower in vaccinated people with at least one chronic disease (OR 0.30 (95% CI 0.12–0.76)) compared to those without chronic disease. The odds of having antibodies were significantly higher in people who had received an mRNA vaccine compared to those who received an adenoviral-vectored vaccine (OR 5.10 (95% CI 1.89–13.79)) and in people who had been vaccinated against influenza in 2020–2021 (OR 2.62 (95% CI 1.06–6.49)) compared to those who are not vaccinated against influenza. All variables found to be significantly associated with having anti-SARS-CoV-2 antibodies in the univariate analyses were modeled in a multivariable model. After adjustment for confounding, the three same variables remained significantly associated with having anti-SARS-CoV-2 antibodies.

3.3.2. Unvaccinated Population

Not surprisingly, the prevalence of anti-SARS-CoV-2 antibodies is lower among the unvaccinated. Interestingly, only 68.7% of the unvaccinated people with one or more positive COVID-19 tests and 11.1% without a positive COVID-19 test presented antibodies. From the univariate analyses, it appears that the odds of being a woman (OR 1.46 (95% CI 1.01–2.12)), having a non-O blood type (OR 1.83 (95% CI 1.11–3.01)), having a close contact with a COVID-19 positive person (OR 3.28 (95% CI 2.08–5.17)), and having one or more positive COVID-19 tests (OR 17.62 (95% CI 10.16–30.57)) were positively associated with having anti-SARS-CoV-2 antibodies. Being a smoker (OR 0.36 (95% CI 0.18–0.72)) was negatively associated with having anti-SARS-CoV-2 antibodies. No association between the presence of anti-SARS-CoV-2 antibodies and age, region of residence, level of education, number of persons in the household, being a healthcare worker or not, presence of a chronic disease, being overweight or obese, having a rhesus type, having received an influenza vaccine, or following the preventive measure could be established. As for the analyses in the vaccinated population, all variables found to be significantly associated with having antibodies in the univariate analyses were modeled in a multivariable equation. After adjustment for confounding, results showed that having a non-O blood type (OR 2.00 (95% CI 1.09–3.67)) and having one or more positive COVID-19 tests (OR 11.04 (95% CI 4.69–26.02)) remained positively associated with having antibodies.

4. Discussion

Results from the SalivaHIS study, conducted when the vaccination campaign in Belgium was running at full speed (between March and August 2021), found that the prevalence of anti-SARS-CoV-2 antibodies among the general population in Belgium increased
significantly from 25.2% in the first period (March 2021, week 13–14) to 78.1% in the last period (July 2021, week 26–27). A previous study, conducted after the first national lockdown in Belgium, showed a seroprevalence of 4.2% in October 2020 [22]. Furthermore, just before the launch of the vaccination campaign in January 2021, seroprevalence studies among Belgian blood donors [12] and primary health care workers [12] described a seroprevalence of 18.7% and 15.1%, respectively. The overall higher seroprevalence in our study mainly reflects the success of the ongoing vaccination campaign. However, also among the unvaccinated population we found a substantially increased seroprevalence compared to October 2020. This shows that there has been an important virus circulation in Belgium during the autumn of 2020, which is compatible with the second wave during this period, and the third wave (March–April 2021). It is remarkable that the seroprevalence in the unvaccinated population remained quite stable (around 17%) between 27 March (start week 13) and 27 June (end week 25). No apparent reason can be found for the sharp increase in the seroprevalence between week 24–25 and week 26–27. This increase is not significant and probably an artefact since the number of saliva samples for this last study period was rather low. Regarding seroprevalence among the vaccinated population, the lower percentage in the beginning of April could be related to the fact that at that time the majority of vaccinated people were older people, with a lower immune response than younger people. During this first period, vaccination in Belgium focused mainly on the older population, people with comorbidities, and health care workers.

SalivaHIS results were compared with other national and international seroprevalence studies that were conducted during the same study period. From these studies it appears that the seroprevalence was 66.1% in the general population in Geneva (July 2021) [8], 89.4% in the general population in England (July 2021) [11], 75.9% among blood donors in Austria (July 2021) [10], and 98.0% (July 2021) among Belgian blood donors [12]. The seroprevalence of 78.1% (July 2021) observed in our study is, thus, relatively consistent with what was found in those other studies [8,10–12]. Of course, these results should be interpreted with caution as the epidemiological situation and the vaccination campaign were different in each country.

Our study provides some interesting results regarding the prevalence of anti-SARS-CoV-2 antibodies among the unvaccinated population in relation to a previous COVID-19 infection. About 18% of the unvaccinated population showed anti-SARS-CoV-2 antibodies. It is important to note that the first data collection of this study took place before the fourth (from 4 October 2021 to 26 December 2021) and fifth (from 27 December 2021 to the end of February 2022) COVID-19 waves in Belgium including the emergence of the Omicron variant [23]. The seroprevalence of 18% is similar to the seroprevalence found at the same time among unvaccinated school children and staff [12] during the same period. It is striking that the seroprevalence among unvaccinated participants with one or more COVID-19 positive tests was only 68.7%. This observation is different than the one identified in a Austrian seroprevalence study among unvaccinated blood donors [10] and in a US study evaluating the prevalence of antibodies among unvaccinated US adults [24]. The lower seroprevalence identified in this study could be explained by the fact that the time since the infection was not considered in our analyses. The infection could have occurred up to 12 months before the saliva collection. Therefore, we can hypothesize a decrease in antibodies after SARS-CoV-2 infection over time. In the Austrian study, they identified a substantial decline in antibody levels during the first 6 months post-infection and a slower decline in the subsequent months [10]. However, a decrease in antibodies after infection is not always observed in other studies [24,25]. A higher seroprevalence among vaccinated people than among people who had a COVID-19 infection could suggest that vaccine-induced antibodies are more effective than natural immunity, but this needs to be interpreted with caution [26]. To investigate this further, the time since vaccination and infection should also be taken into account. Furthermore, in this study as in the Austrian study, around 11% of unvaccinated participants without a previous COVID-19 infection had developed antibodies without knowing it [10]. Unvaccinated people without
a known COVID-19 infection but with anti-SARS-CoV-2 antibodies are either people who had an asymptomatic infection or people who had mild symptoms but remained undiagnosed. They are missed by the epidemiological surveillance which leads to an underestimation of the true number of infected people. This finding emphasizes the added value of seroprevalence studies which provide information on the epidemic, taking into account both diagnosed and undiagnosed cases.

While correlates of protection for SARS-CoV-2 are still not well defined, it is generally accepted that levels of antibodies targeting the Spike RBD of SARS-CoV-2 correlate with a certain level of protection [17, 27]. The results of this study identified some predictive factors associated with the prevalence of anti-SARS-CoV-2 antibodies among the vaccinated and unvaccinated populations.

Although the large majority of the fully vaccinated population in our study had anti-SARS-CoV-2 antibodies, 5% remained non-responders after vaccination. Few data are available on the factors associated with the absence of anti-SARS-CoV-2 antibodies following vaccination against COVID-19. Therefore, an individual’s response after vaccination cannot be predicted yet. Our results found that the seroprevalence was significantly lower in participants with at least one chronic disease, even after adjustment with covariates. Other research is needed to explore if this could be related to a lower immune response of people with certain chronic diseases. Some preliminary results support the fact that the presence of anti-SARS-CoV-2 antibodies could be lower among a population subgroup with chronic diseases and particularly in the immunocompromised population [5, 28]. Unfortunately, the exploration of more in-depth data in this study is limited by small numbers of observations among the different chronic diseases. Since the people with at least one chronic disease are considered a priority for vaccination in Belgium, the number of days since last vaccination could influence seroprevalence among these people. However, in this study, there is no difference in number of days since last vaccination between people with and without a chronic disease (OR: 1.00 (95% CI 0.99–1.02), p-value: 0.49). A higher seroprevalence was associated with being vaccinated against influenza during the previous vaccination season and being vaccinated with an mRNA vaccine compared to an adenoviral-vectored vaccine. Although the potential role of influenza vaccination on COVID-19 outcomes is still unclear, some other studies underlined a negative association between SARS-CoV-2 infection and having been previously vaccinated against the flu [29, 30]. However, further research is needed to understand the protective role of a previous influenza vaccination. With respect to the disparity in antibody presence between the two types of vaccines, other studies have also shown a more consistent and higher presence of anti-SARS-CoV-2 antibodies in people who received an mRNA vaccine type [5, 31].

Initial evidence, although often contradictory, shows that unvaccinated people with blood type A are at higher risk of being infected with SARS-CoV-2, while the risk is decreased in people with blood type O [31–33]. In line with the first evidence, results of this study show that people with blood types A, B, or AB are positively associated with the presence of antibodies. However, while some studies have shown an association between SARS-CoV-2 infection and the rhesus-positive blood type [32–34], these results are not confirmed in the present study. The strong association between a prior infection with SARS-CoV-2 and a higher presence of anti-SARS-CoV-2 antibodies is in line with what can be expected and consistent with the literature [10, 24]. In contrast to findings from other studies, the results of our study, after adjustment for cofounding factors, did not highlight a significant association between the presence of anti-SARS-CoV-2 and different risk factors such as age [8, 10, 35], sex [35, 36], overweight [10, 35], or smoking status [10].

Some limitations to this study need to be highlighted. First, the presence of antibodies was determined by a salivary test and not by a regular serum test. Here, a salivary test was chosen because it is a non-invasive method for comprehensive determination of the presence of vaccine- or natural-induced SARS-CoV-2 antibodies, and facilitates large-scale serosurveillance to assess population seropositivity. The use of a salivary test, although less sensitive than a serum test, is generally accepted for serosurveillance studies and has
been used and validated in other countries [18] and for other pathogens. Unfortunately, the multiplex ELISA test which would allow to make such a distinction was not validated for use in saliva; hence, it was not possible to determine in this study whether vaccinated people with a positive test result had been infected or not. Furthermore, the fact that the higher prevalence among people previously vaccinated against influenza and among people vaccinated with an mRNA vaccine, observed in studies based on blood samples, is also captured by our study confirms the validity of a saliva-based seroprevalence study.

Second, saliva was self-collected by the participants with the Oracol device. Despite an information leaflet and instruction video on how to collect the sample, 17% of the samples contained too little saliva to be analyzed. We have, however, no reason to believe that the participants for whom no saliva result was available, systematically differed from those for whom a result was available. Third, although performance characteristics of the WANTAI SARS-CoV-2 RBD IgG ELISA were good (in house protocol, sensitivity of 91.8% and a specificity of 98.91% in adults), an information bias due to false negative or false positive test results cannot be excluded. Furthermore, the saliva SARS-CoV-2 RBD IgG ELISA returns a semi-quantitative result according to a predefined threshold but did not allow to define a quantitative level of antibodies. Fourth, the required sample size of 1200 individuals per region estimated in the research protocol was not reached because the participation rate was lower than anticipated. However, the net obtained sample was still acceptable for seroprevalence estimates with reasonable precision. A major strength of this study lies in the fact that the SalivaHIS sample was a population-based sample and post-stratification weights were calculated with the aim to produce estimates which were as representative as possible of the total Belgian population. However, participation in the SalivaHIS study was voluntary and although the application of weights ensured that the weighted distributions of the sample by age group, gender, and region matched exactly these distributions in the Belgian population, this could not exclude a selection bias resulting from a differential participation rate within different population groups.

The results obtained in the present and in other studies [37,38] confirm the link between the presence of antibodies and vaccination. However, knowledge on the underlying mechanism explaining the protective role of anti-SARS-CoV-2 antibodies, vaccine- or infection-induced, on infection, reinfection, or disease status remains scarce [39,40]. The emergence of SARS-CoV-2 variants of concern demonstrates that variation in transmissibility, severity, and immune escape potentially underscore the need for additional population level studies evaluating the protective role of variant-specific antibodies [38,39]. Moreover, the finding that anti-SARS-CoV-2 antibodies rapidly wane over time [40–43] drastically impacts the risk of (re)-infection and transmission. Therefore, 3 and 6 month follow-up data from our SalivaHIS cohort will allow an in-depth exploration of the determinants of seroconversion as well as the role of anti-SARS-CoV-2 antibodies on infections and their severity.

5. Conclusions

While the vaccination campaign was going full speed in Belgium, a large increase in the presence of anti-SARS-CoV-2 antibodies has been observed in the general population. The prevalence of antibodies was above 90% among the vaccinated population. Nearly one fifth of the unvaccinated people presented antibodies and this seroprevalence was relatively stable over the time period. Only 68.7% of previously infected, unvaccinated subjects presented antibodies. This study provides a better understanding of the acquired immunity against SARS-CoV-2 among the Belgian population. However, the presence of antibodies and potential correlates of protection against future SARS-CoV-2 infections is still unclear, specifically with emerging variants. A better understanding of the role of antibodies in the protection from the disease is necessary.

Author Contributions: Conceptualization, J.V.d.H., N.V.d.H., L.G. and V.L.; methodology, J.V.d.H.; software, J.V.d.H. and V.L.; formal analysis, J.V.d.H. and V.L.; investigation, J.V.d.H., N.V.d.H., L.G. and V.L.; resources, I.R., C.B. and I.D.; data curation, J.V.d.H. and V.L.; writing—original draft
preparation, J.V.d.H., N.V.d.H. and V.L., writing—review and editing, all authors.; visualization, J.V.d.H., N.V.d.H. and V.L.; supervision, J.V.d.H., E.D.; project administration, J.V.d.H., N.V.d.H. and V.L.; funding acquisition, J.V.d.H. All authors have read and agreed to the published version of the manuscript.

**Funding:** This study was funded by Sciensano, the Belgian institute of public health, Brussels, Belgium. Sciensano was involved in all stages of the study, from conception and implementation to analysis and reporting.

**Institutional Review Board Statement:** The study was approved by the Medical Ethics Committee of the University Hospital Ghent (reference: BC-09362).

**Informed Consent Statement:** Written informed consent was obtained from all participants before enrolment in the study. To guarantee confidentiality, study laboratory results and questionnaires were pseudonymized using unique study codes.

**Data Availability Statement:** Data are available on reasonable request. The statistical codes that support the findings of this study are available from the corresponding author on reasonable request.

**Acknowledgments:** We would like to thank the administrative team for their administrative, logistic, and IT support. We would like also to thank the staff from Statbel for their collaboration in the implementation of the fieldwork of this study. Finally, we are very grateful to all participants of the SalivaHIS study.

**Conflicts of Interest:** The authors declare no conflict of interest.

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