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Long-Term Assessment of the Persistence of Anti-SARS-CoV-2 Antibodies in Vaccinated Healthcare Workers in the Republic of Congo

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Abstract

Introduction: Vaccination is a key strategy in the fight against the COVID-19 pandemic. The objective of this study is to evaluate the duration of vaccine protection through the persistence of anti-SARS-CoV-2 antibodies for up to 49 months in vaccinated healthcare workers. **Methods**: A cross-sectional study was conducted in healthcare facilities in Brazzaville. Serum samples were collected from healthcare workers and analyzed in the laboratory to measure the concentration of anti-S, anti-RBD, and anti-N antibodies using the MagPlex microsphere multiplex immunoassay method. The data were analyzed using R software, version 4.5.1. **Results**: The study included a total of 141 vaccinated healthcare workers, of whom 75.7% were female. The median age was 42.3 years (Q1 = 32 years, Q3=50 years), with a predominance of nurses (50.7%). The mean time since the last vaccine dose was 3.57 ± 0.43 years. The mean antibody concentrations were 13,353 IU/mL for anti-S (min = 14; max = 38,660), 33,224 IU/mL for anti-RBD (min = 1,026; max = 44,471), and 21,706 IU/mL for anti-N2 (min = 402; max = 42,839). The seroprevalence of anti-S antibodies (S_+) was 83.0%. No significant differences were observed based on vaccine type, booster dose administration, or time elapsed since vaccination. **Conclusion**: This study highlights a high persistence of anti-SARS-CoV-2 antibodies up to 49 months after vaccination among healthcare workers in Brazzaville. None of the factors studied—vaccine type, booster dose, or time since vaccination—significantly influenced this seroprevalence. These findings suggest a potentially long-lasting post-vaccination immunity and underscore the importance of longitudinal monitoring to better understand the

durability of immune protection over time.

Keywords

COVID-19, Vaccination, Anti-SARS-CoV-2 Antibodies, Immune Persistence, Healthcare Workers

1. Introduction

The global health crisis caused by the novel virus SARS-CoV-2 had an unparalleled impact on the world. In response to this crisis, a significant mobilisation of scientific and financial resources has enabled the rapid development of several vaccines. These include those from Moderna, Pfizer, Johnson & Johnson, Sinopharm, Sputnik V, Sputnik Light, and AstraZeneca [1]-[4]. Despite their proven effectiveness in reducing the severity of clinical forms, hospitalisations, and mortality [5] [6], vaccines have sparked controversy, fuelled in particular by misinformation and concerns about the speed of their development [7] [8]. These tensions had a significant impact on vaccination coverage, particularly in African countries [9]. Most studies have focused on the assessment of short-term vaccine immunity, typically within six months of vaccination. However, recent studies have emphasised the importance of long-term data collection. In Bangladesh, a longitudinal study demonstrated a 50% decrease in anti-S antibodies one year after vaccination [10]. In China, a significant decrease in neutralising antibodies was observed within 180 days of the booster vaccination, particularly against the Delta and Omicron variants [11]. In the United States, a study noted persistent anti-S antibodies but increased seronegativity in elderly people or those with comorbidities [12]. In sub-Saharan Africa, seroprevalence rates vary widely (20% - 70%), as observed in Kenya and Nigeria, but these studies have often been short-term [13] [14]. The lack of longitudinal data is a significant challenge in the development of suitable vaccination policies, especially for high-risk groups such as healthcare workers.

In the Republic of Congo, data are even more limited. A national study conducted in February 2022 by Ndziessi *et al.* reported a seroprevalence of 48.2% among adults, mainly among those who had been vaccinated, 24 months after the first confirmed case [15]. However, long-term data remain insufficient. The present study aims to evaluate the persistence of anti-SARS-CoV-2 antibodies (anti-S, anti-RBD, and anti-N) up to 49 months after vaccination in healthcare workers in Brazzaville, in an African context. Its purpose is to provide empirical data to better understand the durability of the humoral response and guide booster vaccination strategies in at-risk populations.

2. Patients and Methods

2.1. Study Design and Population

A cross-sectional analytical study was conducted. Data were collected from 13

February to 28 June 2025, allowing for the collection of post-vaccination information. The target population included healthcare workers, both those directly involved in patient care (doctors, nurses, nursing assistants) and non-clinical staff (administrative, technical, and logistical personnel). The exact earliest and latest vaccination dates were respectively March 27, 2021, and October 1, 2022. These professionals were selected due to their increased risk of infection through regular contact with patients suffering from various pathologies, including SARS-CoV-2. The study was conducted in the main public referral hospitals in Brazzaville, namely Makélékélé Basic Hospital, Bacongo Basic Hospital, Talangaï Referral Hospital, the Sino-Congolese Friendship Hospital in Mfilou, as well as several peripheral health centres, ensuring a diverse representation of healthcare settings. The inclusion criteria focused on complete vaccination against COVID-19 administered in the Republic of Congo, as well as membership of the healthcare staff of the selected establishments. Those who did not meet the necessary criteria or declined to participate were excluded from the study. Participants were recruited using non-probability convenience sampling.

2.2. Sample Collection and Antibody Assay

Blood samples were collected in dry tubes. Following centrifugation, 1 µL of plasma was diluted in 400 µL of an internally prepared buffer composed of 1% phosphate-buffered saline (PBS), 5% heat-inactivated bovine serum albumin (BSA), and 0.2% Tween 20, all diluted in 1 liter of sterile distilled water. The remaining serum was then aliquoted and stored in a −80˚C freezer for future analysis. The measurement of anti-SARS-CoV-2 antibodies was conducted using a multiplex immunoassay on MagPlex microspheres, employing Luminex xMAP™ technology. This method offers good sensitivity and specificity, allowing for the simultaneous detection of antibodies directed against three distinct antigens of the virus: the spike protein (S), the Receptor Binding Domain (RBD), and the nucleocapsid (N2) [16]-[20]. MagPlex microspheres were coupled to viral antigens using an amine coupling kit (Bio-Rad Laboratories) in accordance with the manufacturer's protocol. Readings were performed on a Magpix system (Luminex), with a minimum of 100 events acquired per sample. The results were expressed as Median Fluorescence Intensity (MFI), which reflects the number of specific antibodies bound. Biological analyses were carried out at the National Public Health Laboratory.

2.3. Serological Positivity Threshold for SARS-CoV-2 Antibodies

Samples were considered seropositive for SARS-CoV-2 antibodies if the Median Fluorescence Intensity (MFI) values for both the Spike (S) and Receptor Binding Domain (RBD) exceeded the mean MFI of pre-pandemic (negative control) samples by more than three standard deviations [17]. This statistical approach minimizes false positives by ensuring precise discrimination between negative and pos-

itive serum samples. An S^+ classification was established when both S and RBD were positive [19]. We estimated seroprevalence, the proportion of individuals who were S^+.

2.4. Study Variables

The variables analyzed included:

- Demographic variables: age (in years), gender (male/female), marital status (single/in a relationship), professional rank (doctor, nurse, midwife, biologist, administrative staff, other), and level of education (secondary, higher).
- Clinical variables: high blood pressure, diabetes, hepatitis, stroke, HIV, history of COVID-19 (all coded as yes/no).
- Vaccination variables: vaccine name (Sinopharm, Sputnik Light, Sputnik V, Johnson & Johnson), vaccination delay, booster doses (yes/no).
- Biological variables: anti-S, anti-RBD, and anti-N2 antibody titers.

2.5. Statistical Analysis

Qualitative data were presented in frequency and proportion tables, while quantitative variables were expressed as means, standard deviations, medians, and quartiles. Comparisons of proportions were performed using Pearson's Chi^2 test or Fisher's exact test, depending on the conditions. The significance threshold was set at $p < 0.05$. All analyses were performed using R software, version 4.5.1.

2.6. Ethical Considerations

The protocol of this study was approved by the Health Sciences Research Ethics Committee (CERSSA) under number 137-24/MERSIT/DGRST/CERSSA/-24. The study complied with international ethical principles, including the informed consent of participants, as well as the confidentiality and anonymity of data. Each participant was informed of the objectives, the terms and conditions of participation, and their right to withdraw without any consequence. The data were anonymised and secured to ensure the protection of personal information.

3. Results

3.1. Main Characteristics of the Study Population

Of the 141 participants, the majority were female (74.5%) and aged between 18 and 44 years (57.4%). The median age was 42.3 years (Q1 = 32 years, Q3 = 50 years). Furthermore, it is notable that more than half of the respondents were in a relationship (59.6%) and had a higher level of education (66.7%). In terms of professional rank as in the Table 1, the largest group was nurses (49.6%), followed by administrative staff, midwives, doctors, and biologists (Table 1).

As shown in Table 2, 17.7% of participants had high blood pressure and 5.7% had diabetes. The prevalence of viral hepatitis, HIV infection, and a history of stroke was low, each accounting for 0.7%. Finally, 3.5% of healthcare workers reported a history of SARS-CoV-2 infection.

Table 1. Sociodemographic characteristics of the healthcare workers surveyed.

Variables	Frequency	Percentage
Gender		
Male	36	25.5
Female	105	74.5
Age group (years)		
18 - 44	81	57.4
45 - 65	60	42.6
Marital status		
Single	57	40.4
In a relationship	84	59.6
Level of education		
Secondary	47	33.3
Higher	94	66.7
Professional rank		
Nurse	70	49.6
Administrative staff	26	18.4
Midwife	22	15.6
Doctor	10	7.1
Biologist	6	4.3
Other	7	5.0

Table 2. Main clinical characteristics of the healthcare workers tested.

Variables	Frequency	Percentage
High blood pressure	25	17.7
Diabetes	8	5.7
Hepatitis	1	0.7
HIV	1	0.7
Stroke	1	0.7
History of COVID-19 infection	5	3.5

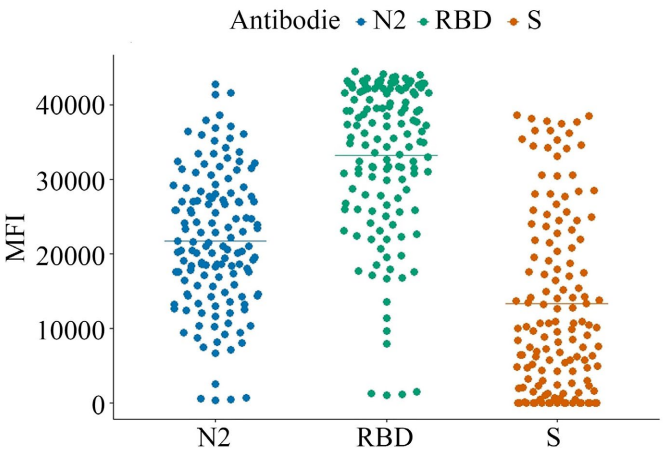
As shown in Table 3, 39.0% of vaccinated healthcare workers had received the Johnson & Johnson vaccine, 27.0% the Sputnik Light vaccine, 25.5% the Sinopharm vaccine, 7.8% the Sputnik V vaccine, and 0.7% the Pfizer vaccine. Regarding booster doses, only 13.5% of participants had received one, while 86.5% had received only the primary vaccination regimen. The time elapsed since the last vaccination was mainly between three and five years for 78.7% of participants, and between two and three years for 21.3%.

Table 3. Vaccine characteristics of health workers.

Variables	Frequency	Percentage
Vaccine name		
Johnson & Johnson	55	39.0
Sputnik light	38	27.0
Sinopharm	36	25.5
Sputnik V	11	7.8
Pfizer	1	0.7
Booster doses		
Yes	19	13.5
No	122	86.5
Vaccination delay (years)		
2 - 3	30	21.3
3 - 5	111	78.7

3.2. Assaying Anti-Spike, Anti-RBD, and Anti-Nucleocapsid Antibodies

As illustrated in Figure 1, the mean concentrations of anti-SARS-CoV-2 antibodies in vaccinated healthcare workers are shown. The mean concentration of anti-spike (anti-S) antibodies was 13,353 IU/mL, with a minimum of 14 IU/mL and a maximum of 38,660 IU/mL. The mean concentration of anti-RBD antibodies was 33,224 IU/mL, with a range from 1,026 IU/mL to 44,471 IU/mL. The mean concentration of anti-N2 antibodies was 21,706 IU/mL, with a range of 402 to 42,839 IU/mL.



RBD: Receptor Binding Domain, **S**: Spike, **N2**: Nucleocapsid Protein, **MFI**: Median Fluorescence Intensity.

Figure 1. Antibody concentration in healthcare workers vaccinated.

As illustrated in Figure 2, the means of antibody concentrations are indicative

of the type of vaccine administered to healthcare professionals. For all vaccines analysed, the highest concentrations of antibodies were observed for anti-RBD, followed by anti-N2.

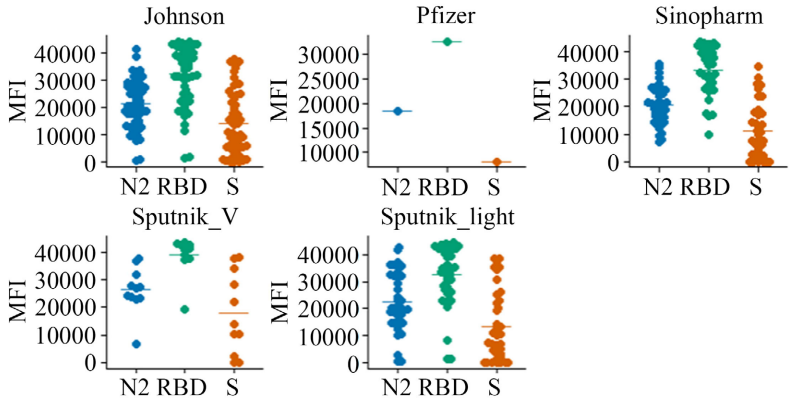


Figure 2. Antibody concentration in vaccinated healthcare workers according to the vaccine.

3.3. Seroprevalence of Antibodies According to Vaccine Characteristics

Applying the defined positivity thresholds, the overall seroprevalence of antibodies (S^+) was 83.0% (117/141). Table 4 examines the distribution of seropositivity according to vaccine type, booster dose administration, and time elapsed since the last injection.

The seroprevalence rates by vaccine received range from 78.9% to 100%, with no statistically significant difference (p = 0.900). This suggests that the different vaccines are equally effective in inducing a detectable long-term humoral response.

The administration of a booster dose did not significantly influence the seroprevalence of SARS-CoV-2 antibodies (78.9% in those who did not receive a booster vs. 83.6% in those who did; p = 0.700). Similarly, no significant difference was observed based on the post-vaccination interval, whether it was between 2 - 3 years or between 3 - 5 as shown in Table 4 (p = 1.000), indicating the relative stability of S^+ antibodies over this period.

Table 4. Seroprevalence of antibodies (S^+) according to vaccine characteristics.

Variables	Frequency	Positive test (S^+)	Seroprevalence (S^+)
Total	141	117	83.0
Vaccine name			
Johnson & Johnson	55	47	85.5
Sputnik light	38	30	78.9
Sinopharm	36	30	83.3
Sputnik V	11	9	81.8
Pfizer	1	1	100.0
P-value			0.900

Continued

Booster doses			
Yes	19	15	78.9
No	122	102	83.6
P-value			0.700
Vaccination delay (years)			
2 - 3	30	25	83.3
3 - 5	111	92	82.9
P-value			1.000

4. Discussion

The objective of this study was to evaluate the duration of vaccine protection through the persistence of anti-SARS-CoV-2 antibodies for up to 49 months in vaccinated healthcare workers. The seroprevalence of antibodies was estimated between 28 and 49 months after the administration of the last vaccine dose.

The results of this study demonstrate that antibodies remain present in vaccinated healthcare workers up to 49 months after the administration of the last vaccine dose. The highest mean concentration was observed for anti-RBD antibodies, followed by anti-N2 antibodies. The seroprevalence of antibodies against the S protein (S^+ profile) was high among vaccinated healthcare workers, reaching 83.0%. This indicates a notable persistence of the immune response even several years after the last dose of vaccine. Our findings align with those reported by Nurjaya *et al.* in Indonesia, who observed antibody persistence in vaccinated healthcare workers beyond 24 months [21]. A study conducted in the Central African Republic (CAR) revealed a high seroprevalence rate among healthcare workers who received their vaccination 12 months after the initial programme [22]. The prolonged persistence of antibodies in vaccinated healthcare workers may be explained by repeated exposure to the virus due to their frequent contact with infected patients, which could promote asymptomatic reinfection. A study conducted by Massala *et al.* in Congo reported an increase in the incidence of COVID-19 over time among vaccinated people, which was more pronounced among healthcare workers [23]. The detection in our study of anti-N2 antibodies in healthcare workers who received non-viral vector vaccines (Johnson & Johnson, Pfizer, Sputnik V, and Sputnik Light), which do not contain the viral nucleoprotein, supports the hypothesis of prior exposure to SARS-CoV-2. This suggests a mixed humoral response, combining vaccine-induced immunity and potentially post-infectious immunity. In fact, the main antigenic target for vaccination is the spike protein. However, vaccines such as Sinopharm are inactivated virus vaccines that trigger the production of anti-N2 antibodies [1] [24]-[26]. Furthermore, anti-N2 antibodies are more effective than anti-spike antibodies at detecting infection [27]. Several studies have shown that the immune response is enhanced in vaccinated individuals who were infected before or after vaccination [22] [28]-[34]. In Austria, the study conducted by Nunhofer *et al.* showed that antibodies result-

ing from natural infection in vaccinated individuals declined over time but could persist for more than a year [35].

This distinction between vaccine-induced and infection-induced antibody responses is particularly relevant in contexts where diagnostic capacity is limited. The Republic of Congo, like many other developing countries, has limited capacity to diagnose the disease. This limitation may be responsible for underestimating the true extent of the disease, as reported by Ndziessi *et al.* in Congo [15]. Consequently, many infections remain undocumented, particularly in cases where patients are asymptomatic or only display mild symptoms. Consequently, there are several infections that have not been documented. This absence of documentation can introduce a confounding bias in the analysis of antibody persistence, as an undiagnosed person may have high antibody levels, not only because of vaccination, but also because of a recent infection. The use of anti-N2 antibody data can partially control for this effect, as the presence of these antibodies indicates natural infection, except in the case of inactivated virus vaccines such as Sinopharm.

However, this study has several strengths. Firstly, it assesses the persistence of antibodies over a long period (49 months). Secondly, the differentiated analysis of antibodies (anti-S, anti-RBD, and anti-N2) allows for a better interpretation of the source of immunity. Thirdly, the local context in Congo is poorly documented, which lends this work additional scientific and contextual value. Fourthly, the Luminex assay is a reliable method for measuring anti-SARS-CoV-2 antibodies. This has been demonstrated by studies conducted in Congo [17] [18] and elsewhere [19] [20] [36].

While the study also has some methodological limitations related to its cross-sectional design, which measures antibodies at a single point in time, thereby making it impossible to track their evolution over time; the small sample sizes for each vaccine type limit the statistical power of the analysis to detect potential differences in seroprevalence between vaccinated groups; the vaccine-induced immunity and infection-induced immunity were not differentiated due to the use of tests that cannot distinguish between antibodies from vaccination and those from natural infection; there is selection bias because the healthcare professionals included are not representative of all vaccinated individuals in Brazzaville; and finally, there is no assessment of the neutralising capacity of the antibodies. A longitudinal follow-up of the same workers would better capture antibody kinetics.

These findings have several implications for public health. It is important to periodically measure post-vaccination immunity among healthcare workers to adapt vaccination strategies, integrate serological surveillance into the surveillance system for detecting asymptomatic infections among healthcare workers, and raise awareness among healthcare workers about the need to maintain preventive measures despite vaccination.

5. Conclusion

This study highlights a high persistence of anti-SARS-CoV-2 antibodies up to 49

months after vaccination among healthcare workers in Brazzaville. None of the factors studied, vaccine type, booster dose, or time since vaccination, significantly influenced this seroprevalence. These findings suggest a potentially long-lasting post-vaccination immunity and underscore the importance of longitudinal monitoring to better understand the durability of immune protection over time.

Authors' Contributions

J. M. P. drafted the protocol, collected and analysed the data, and wrote the draft of the manuscript. L. H. L. drafted the biological methodology and analysed the samples. G. N. and L. H. L. revised the manuscript. G. N., G. E. E. M., F. K. K., and R. F. N. supervised and validated the various stages. J. R. N. A., A. C. N., and J. A. N. proofread the manuscript. All authors contributed to this study.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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