

Research Article

Factors Associated with Post-vaccination Immunity Against COVID-19 in Benin

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Abstract

Introduction: Vaccination remains the primary strategy against COVID-19. However, data may not reflect actual population immunity. This study aims to determine the seroprevalence of post-vaccination immunity against COVID-19 in Benin and identify associated factors. **Methods:** A cross-sectional descriptive and analytical study was conducted from April to September 2023 in 21 hospital centers across Benin's twelve departments and included 3802. Post-vaccination immunity was defined by the presence of anti-SARS-CoV-2 Spike IgG antibodies and the absence of anti-NCP IgG antibodies, using the Euroimmun ELISA test. Data were analyzed using STATA software with multivariate logistics regression. **Results:** Seroprevalence of post-vaccination immunity was 41.56%. In multivariate analysis, factors associated with this immunity were age (adjusted OR=1.56; 95% CI [1.09-2.59]), residence within the former sanitary cordon (aOR=1.34; 95% CI [1.14-3.01]), history of hypertension/cardiovascular disease (aOR=3.25; 95% CI [1.41-4.63]), and history of diabetes (aOR=2.73; 95% CI [1.89-3.93]). **Conclusion:** Post-vaccination immunity is higher among vulnerable groups (elderly, comorbidities) and those in strategic urban zones, reflecting the prioritization of vaccination campaigns. Recommendations are proposed to target younger populations and rural areas to strengthen collective immunity.

Keywords

COVID-19, Post-vaccination Immunity, Age Comorbidities, Benin

1. Introduction

Since its emergence in late 2019, the COVID-19 pandemic caused by SARS-CoV-2 has disrupted global health systems and imposed unprecedented socio-economic challenges [1]. While Europe and the Americas have paid a heavy price in

terms of mortality, Africa has presented an epidemiological profile characterized by case fatality rates lower than initial projections [2, 3]. However, this apparent resilience should not obscure the active circulation of the virus or the persistent

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risk of new epidemic waves linked to the emergence of concerning variants [4]. In this context, vaccination has become the central strategy of the global response to contain viral transmission and reduce the burden on hospitals [5].

In Benin, the national vaccination strategy was deployed as an accelerated campaign despite socio-community vaccine hesitancy [5, 6]. While health authorities have administrative data on the number of doses administered, these indicators remain insufficient to assess the actual protection of the population [6]. Indeed, administrative vaccination coverage can be biased by reporting errors or loss of follow-up, and does not provide information on the actual biological response of individuals [7]. Moreover, immunity against SARS-CoV-2 is a composite phenomenon: it can result from natural infection, vaccination, or a combination of both [8].

The serological distinction between these types of immunity is fundamental for guiding public health policies. Currently available vaccines, particularly those using mRNA or viral vectors, primarily target the virus's Spike (S) protein. Consequently, purely post-vaccination immunity is biologically manifested by the presence of anti-Spike IgG antibodies (Anti-Spike) in the absence of antibodies directed against the nucleocapsid (Anti-NCP), the latter being produced only in response to infection with the whole virus [9, 15]. This biological signature allows for the differentiation of vaccine-protected individuals from those who have contracted the disease [10].

Several sero-epidemiological studies have been conducted in Europe and Asia to assess virus penetration [11, 40], and data specific to West Africa warrant further investigation [12]. The few available studies have often been limited to overall seroprevalence without distinguishing the origin of immunity [13]. However, understanding the specific determinants of post-vaccination immunity is crucial. This allows not only for evaluating the effectiveness of vaccine targeting during the pandemic, but also for identifying sociodemographic groups that have remained outside the vaccination network [14].

This study aims to address this knowledge gap by analyzing the seroprevalence of strictly post-vaccination immunity (Anti-S positive / Anti-NCP negative) in Benin. Using a rigorous methodology with validated ELISA tests [15], the objective is to identify the sociodemographic and medical factors associated with this acquired immunity, in order to provide policymakers with tangible evidence to optimize future vaccination campaigns and anticipate booster dose needs.

2. Materials and Methods

2.1. Study Design, Type and Duration

This was a cross-sectional study with descriptive and analytical aims. Data collection took place over a period of six (06) months, from April to September 2023. The study was conducted in selected hospital centers distributed across the twelve departments of Benin, ensuring national representa-

tiveness of the hospital health context.

2.2. Study Population and Sampling

The study population consisted of adults and children of all ages who had attended the hospitals identified for recruitment during the study period.

Selection criteria

Subjects meeting the following criteria were included in this study:

- 1). Having attended one of the hospitals involved in the study during the data collection period.
- 2). Having given informed consent to participate in the study (or consent from parents/legal guardians for minors).

The following were excluded from the study:

- 1). The subjects who refused to participate.
- 2). Subjects whose blood samples proved unusable in the laboratory.
- 3). Subjects vaccinated less than one month before the study period, in order to ensure that the measured immune response is established.

The sampling procedure was carried out in two distinct stages (two-stage sampling):

- 1). Sampling of health centers: a probabilistic selection using simple random sampling was carried out from the database of health facilities in each department. In total, 21 hospitals were selected across the country's 12 departments.
- 2). Population sampling: Within each selected center, the selection of participants was carried out by a non-probabilistic method (convenience sampling) based on the active patient roster.

At the end of the recruitment process, 3802 people were included in the study.

2.3. Data Collection

Sociodemographic and clinical data were collected via a structured questionnaire and a standardized data collection form.

The biological (serological) data were obtained by analyzing blood samples using the Euroimmun anti-SARS-CoV-2 ELISA IgG test. This enzyme-linked immunosorbent assay allows for the qualitative in vitro determination of human IgG antibodies directed against SARS-CoV-2 (serum/plasma). The kit's reliability is demonstrated by a sensitivity of 94.4% and a specificity of 98.5% [15].

Primary data collection involved reviewing medical records, consultation notes, and laboratory reports. For hospitalized patients, a face-to-face interview was conducted to complete the questionnaire. Outpatients were contacted by telephone to obtain their consent and administer the questionnaire.

Blood samples (EDTA or citrate tubes) were transported to the National Laboratory for Viral Hemorrhagic Fevers (LNFVH) under cold chain conditions and then stored between -80°C and 4°C before analysis. Interpretation was based on the ratio (sample OD / calibrator OD). A ratio < 0.8 is negative; between 0.8 and 1.1 is equivocal; ≥ 1.1 is positive.

2.4. Study Variables

Dependent variable

The variable of interest is post-vaccination immunity, biologically defined by the simultaneous presence of the Anti-Spike IgG antibody and the absence of the Anti-NCP IgG antibody (specific to natural infection). Anti-Spike (+) and Anti-NCP (-). The comparator group ("No Post-vaccination immunity") is defined as any participant not meeting this strict profile, including both seronegative (naïve) individuals and those with serological evidence of prior natural infection (Anti-NCP +).

Independent variables

- 1). Sociodemographics: Sex, age, place of residence (urban, semi-rural, rural), residence within sanitary cordon (high surveillance zone at the beginning of the pandemic), monthly income, socioeconomic level, education level, occupation, ethnicity, household size, religion, geographic department.
- 2). Medical history: Hypertension and cardiovascular diseases, diabetes, sickle cell disease, kidney failure.
- 3). Related to COVID-19: History of infection (personal or family), perception of fear (scale 0-10).
- 4). Declared vaccination status: Vaccinated, unvaccinated, opposed to vaccination.

2.5. Data Processing and Analysis

The statistical analysis was performed using STATA® software.

- 1). Descriptive analysis: Qualitative variables were described by frequencies and percentages, and quantitative variables by means and standard deviations (SD).
- 2). Univariate analysis: Associations between independent variables and post-vaccination immunity were tested using the Pearson's chi-square test (percentages) or the Student's t-test (means). Statistical significance was defined as $p < 0.05$. No post-hoc corrections for multiple testing were applied at the univariate level.
- 3). Multivariate analysis: Variables with a $p \leq 0.20$ in univariate analysis were included in a binary logistic regression model. The final model was obtained using a backward stepwise procedure to identify associated independent factors (adjusted ORs with 95% CI).

2.6. Ethical Considerations

The study adhered to the principles of medical ethics. Research authorization was obtained from the hospital authori-

ties. Informed consent (written or oral) was obtained from each participant. Anonymity and data confidentiality were strictly guaranteed throughout the process.

3. Results

3.1. Seroprevalence of Post-vaccination Immunity

Of the 3802 participants included in the study, serological analysis revealed that 1580 individuals had an immunological profile corresponding to strict post-vaccination immunity (Anti-Spike positive and Anti-NCP negative).

Thus, the seroprevalence of post-vaccination immunity in the study population was 41.56%.

Post-vaccination immunity

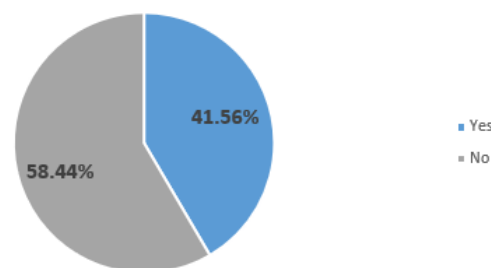


Figure 1. Seroprevalence of post-vaccination immunity.

3.2. Description of Subjects with Post-vaccination Immunity

Analysis of the sociodemographic characteristics of the 1580 immunized subjects shows a female predominance (55.26%). The mean age of these subjects was 35.0 ± 18.0 years. The most represented age group was 20-40 years (34.49%), followed by 5-20 years (25.82%).

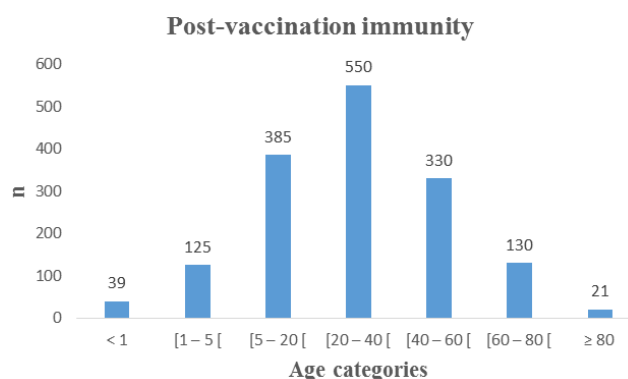


Figure 2. Distribution by age group.

Regarding residence, 40% of immunized individuals lived in urban areas, and 80% resided outside the former sanitary cordon. The distribution of immunized individuals varied by

department. The departments of Atlantique (14.24%), Littoral (13.99%), and Alibori (13.48%) had the highest proportions of immunized individuals.

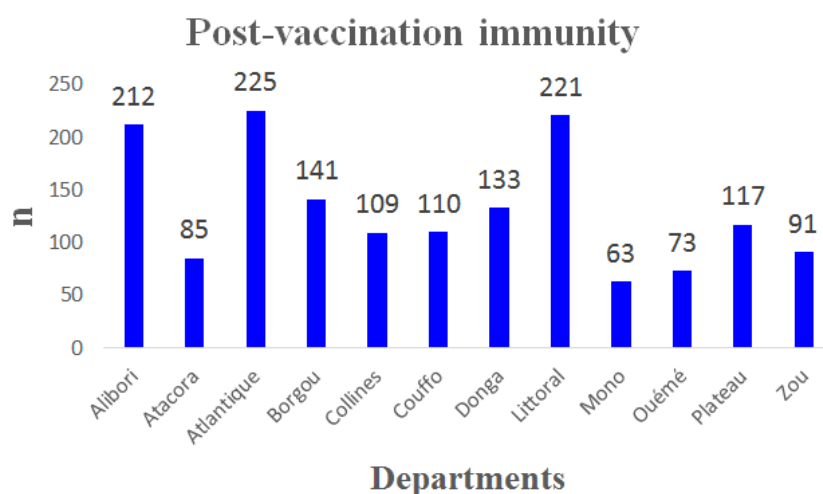


Figure 3. Geographical distribution.

On a socio-economic level, the majority had a medium (35%) or low (30%) level.

Regarding medical history, among subjects with post-vaccination immunity, 30% had hypertension or cardi-

ovascular disease, and 9% were diabetic. Concerning self-reported vaccination status, 28.73% reported being vaccinated, while 16.71% had a vaccination record confirmed by the interviewer.

3.3. Factors Associated with Post-vaccination Immunity

Table 1. Univariate analysis of sociodemographic characteristics associated with post-vaccination immunity.

Variables	Post-Vaccination Immunity, Yes (n=1580)	Post-Vaccination Immunity, No (n=2222)	p-value
Sex			0.31
Male	698 (44.2%)	1003 (45.1%)	
Female	882 (55.8%)	1219 (54.9%)	
Age (years)			< 0.001
Mean (SD)	38.2 ± 15.1	32.5 ± 16.9	
Age Group (years)			0.002
< 1	39 (2.5%)	60 (2.7%)	
1 – 4	125 (7.9%)	179 (8.1%)	
5 – 19	385 (24.4%)	596 (26.8%)	
20 – 39	550 (34.8%)	762 (34.3%)	
40 – 59	330 (20.9%)	419 (18.9%)	
60 – 79	130 (8.2%)	174 (7.8%)	
≥ 80	21 (1.3%)	32 (1.4%)	
Living environment			0.08
Urban	608 (38.5%)	913 (41.1%)	

Variables	Post-Vaccination Immunity, Yes (n=1580)	Post-Vaccination Immunity, No (n=2222)	p-value
Semi-rural	456 (28.9%)	685 (30.8%)	0.002
Rural	516 (32.7%)	624 (28.1%)	
Residence within sanitary cordon			
Yes	380 (24.1%)	380 (17.1%)	
No	1200 (75.9%)	1842 (82.9%)	0.65
Monthly income (CFA Francs XOF)			
Refusal to answer	61 (3.9%)	87 (3.9%)	
< 50,000	458 (29.0%)	645 (29.0%)	
50,000 – 150,000	616 (39.0%)	867 (39.0%)	0.51
150,000 – 250,000	316 (20.0%)	444 (20.0%)	
> 250,000	129 (8.2%)	179 (8.1%)	
Socioeconomic level			
Very low	237 (15.0%)	333 (15.0%)	0.15
Low	474 (30.0%)	667 (30.0%)	
Average	553 (35.0%)	778 (35.0%)	
High	237 (15.0%)	333 (15.0%)	
Very high	79 (5.0%)	111 (5.0%)	0.41
Education level			
Not enrolled	506 (32.0%)	711 (32.0%)	
Primary	442 (28.0%)	623 (28.0%)	
Secondary	395 (25.0%)	556 (25.0%)	0.78
Higher	237 (15.0%)	333 (15.0%)	
Occupation			
Unemployed	316 (20.0%)	444 (20.0%)	
Student	237 (15.0%)	333 (15.0%)	0.91
Factory worker	253 (16.0%)	355 (16.0%)	
Merchant	380 (24.1%)	532 (23.9%)	
Civil Servant / Manager	394 (24.9%)	558 (25.1%)	
Ethnic group			0.35
Dendi	300 (19.0%)	422 (19.0%)	
Bariba	300 (19.0%)	422 (19.0%)	
Fon	442 (28.0%)	623 (28.0%)	
Goun	332 (21.0%)	466 (21.0%)	0.91
Other	206 (13.0%)	289 (13.0%)	
Household size			
≥ 5	1027 (65.0%)	1444 (65.0%)	
< 5	553 (35.0%)	778 (35.0%)	0.35
Religion			

Variables	Post-Vaccination Immunity, Yes (n=1580)	Post-Vaccination Immunity, No (n=2222)	p-value
Christian	695 (44.0%)	978 (44.0%)	
Traditional	111 (7.0%)	155 (7.0%)	
Muslim	711 (45.0%)	1000 (45.0%)	
Other	63 (4.0%)	89 (4.0%)	

Univariate analysis identified several factors potentially associated with post-vaccination immunity. Regarding sociodemographic characteristics (Table 1), the mean age was significantly higher in immunized subjects (38.2 years) compared to non-immunized subjects (32.5 years) ($p < 0.001$). Age group was significantly associated, particularly the 40-60 age group, as was residence within the sanitary cordon

($p = 0.002$). Sex, education level, occupation, ethnicity, and religion were not statistically associated.

Analysis of medical history (Table 2) revealed a very strong association between post-vaccination immunity and the presence of comorbidities. Diabetes ($p = 0.001$) and hypertension ($p < 0.001$) were significantly more frequent in immunized subjects.

Table 2. Univariate analysis of medical history.

Variables	Post-Vaccination Immunity, Yes (n=1580)	Post-Vaccination Immunity, No (n=2222)	p-value
Diabetes			0.001
Yes	127 (8.0%)	215 (9.7%)	
No	1453 (92.0%)	2007 (90.3%)	
Hypertension and other CVD			< 0.001
Yes	380 (24.1%)	761 (34.2%)	
No	1200 (75.9%)	1461 (65.8%)	
Sickle cell disease			0.68
Yes	79 (5.0%)	111 (5.0%)	
No	1501 (95.0%)	2111 (95.0%)	
Kidney failure			0.15
Yes	63 (4.0%)	89 (4.0%)	
No	1517 (96.0%)	2133 (96.0%)	

History of COVID-19 and geographic distribution showed no significant association with post-vaccination immunity in univariate analysis.

Multivariate analysis

After adjustment in the logistic regression model, four factors remained independently associated with post-vaccination immunity (Table 3).

Advanced age increases the risk of developing

post-vaccination immunity (adjusted OR = 1.56; 95% CI [1.09–2.59]). Residence within the quarantine zone is a contributing factor (adjusted OR = 1.34; 95% CI [1.14–3.01]). Comorbidities play a major role: individuals with a history of hypertension (adjusted OR = 3.25; 95% CI [1.41–4.63]) and diabetes (adjusted OR = 2.73; 95% CI [1.89–3.93]) have a significantly higher likelihood of developing post-vaccination immunity.

Table 3. Multivariate analysis of factors associated with post-vaccination immunity.

Explanatory variables	Post-vaccination immunity	
	adjusted OR [95% CI]	p
Age	1.56 [1.09-2.59]	0.01
Residence within the sanitary cordon (yes)	1.34 [1.14-3.01]	0.006
History of hypertension and other CVD (yes)	3.25 [1.41-4.63]	0.000
History of diabetes (yes)	2.73 [1.89-3.93]	0.000

4. Discussion

This cross-sectional study, conducted with 3,802 participants across Benin, offers a unique perspective on the immunological status of the post-pandemic hospital population. With a post-vaccination immunity seroprevalence (defined by the exclusive presence of anti-spike antibodies) of 41.56%, our results reveal substantial vaccine-induced immune coverage, although not optimal for ensuring robust herd immunity against highly transmissible variants [16]. This rate, higher than the simple vaccination declarations often reported in opinion polls, underscores the crucial importance of biological surveillance compared to self-reported data [17]. Compared to regional data, this rate falls within an intermediate range compared to studies conducted in Nigeria or Togo, reflecting the ongoing efforts of Beninese health authorities despite a regional context of vaccine hesitancy [18, 19].

Multivariate analysis highlighted major determinants of this immunity, foremost among them comorbidities. Our results indicate that diabetic and hypertensive subjects are respectively 2.73 and 3.25 times more likely to have post-vaccination immunity than the general population [20]. This finding illustrates a health-seeking behavior: patients with chronic diseases, aware of their vulnerability to severe forms of COVID-19 documented by the CDC and WHO [21], adhered more closely to vaccination recommendations [22]. In Benin, this adherence was reinforced by targeted communication to these at-risk populations during follow-up consultations, thus validating the effectiveness of integrating COVID-19 vaccination into the care pathway for chronically ill patients, as shown in several studies [23-25].

Age also proved to be a strong predictor (OR = 1.56). The increased prevalence of vaccine antibodies in older subjects reflects adherence to the vaccination prioritization established by the Beninese government, aligned with international guidelines [26]. Furthermore, the observed lower rate of "strict" post-vaccination immunity (Anti-S+/Anti-NCP-) among younger individuals may be influenced by exposure patterns. Younger, healthier individuals are typically more socially active and thus more prone to natural infection. Since

our study definition excludes individuals with Anti-NCP antibodies (markers of natural infection) from the "post-vaccination immunity" group, this creates a mechanical reduction in the seroprevalence of "vaccine-only" immunity in this demographic, favoring the elderly who may have shielded more effectively. However, this result should be interpreted with caution from an immunological perspective: while coverage is good, the scientific literature suggests that the humoral response (neutralizing antibody levels) tends to decline more rapidly in older adults (immunosenescence) [28, 29]. The qualitative presence of antibodies (positive test) does not guarantee indefinite protection against infection, justifying the need to maintain a booster dose policy for this specific age group [27].

Another key finding lies in the geographical disparity linked to the former sanitary cordon. Residents of this strategic zone, which encompassed the major urban economic centers (Littoral, Atlantique, Ouémé) at the beginning of the pandemic, had a significantly higher probability of being immune (OR = 1.34) [30]. This result highlights the impact of structural and environmental determinants on health. The sanitary cordon zone benefited from a denser healthcare system, early vaccine availability, and intense media exposure to prevention messages [31, 32]. Conversely, rural or peripheral areas appear to have received less coverage, underscoring the challenges of territorial equity in access to public health interventions [33].

It is also striking to note the absence of a significant association between post-vaccination immunity and socioeconomic status or educational level in our multivariate model. Contrary to numerous observations in Western countries where social status is a strong predictor of vaccine acceptance [34, 35], it appears that in Benin, risk perception (related to comorbidities) and geographical accessibility (sanitary cordon) prevailed over traditional social determinants. This could also suggest that the influence of community and religious leaders, which transcends social classes, played a key role in vaccine acceptance across all strata of society [36].

However, our study has inherent limitations. First, the use of non-probabilistic convenience sampling in hospital settings limits the generalizability of our results to the broader general

population. Hospital attendees may have different health-seeking behaviors or underlying health conditions compared to the general community, potentially introducing selection bias [37]. Second, the absence of a formal sample size calculation prior to recruitment means the study power was not optimized for all sub-group analyses. Furthermore, our definition of the comparator group ("No Post-vaccination immunity") is heterogeneous. It includes both immunologically naïve individuals and those with markers of prior natural infection (Anti-NCP positive). This introduces a potential confounding factor, as the "non-immune" group is not strictly a "non-exposed" group. Consequently, our study specifically assesses "vaccine-only" immunity and does not capture the full scope of protection provided by hybrid immunity (vaccination plus infection), which recent literature suggests may offer robust protection [38, 39]. Additionally, the definition of the comparator group (which includes both naïve and naturally infected subjects) introduces a potential confounding factor regarding exposure risk, as discussed above. Finally, due to the cross-sectional design and lack of data on the "time since vaccination," we could not assess antibody waning kinetics or correlate seropositivity with the duration of protection. [40].

5. Conclusion

This study highlights a post-vaccination immunity seroprevalence of 41.6% among the population consulting at hospitals in Benin in 2023. The main determinants of this immunity are advanced age, residence in strategic urban areas, and the presence of comorbidities. These results indicate that the strategy of prioritizing vulnerable individuals has been successful in terms of immunization coverage.

6. Recommendations

Future vaccination strategies should specifically target younger populations and rural areas to bridge the immunity gap. Booster doses should be maintained for at-risk groups (elderly, comorbidities) given the waning of humoral immunity. Surveillance systems should integrate hybrid immunity monitoring to provide a more comprehensive picture of population protection.

Abbreviations

CVD	Cardiovascular Diseases
EDTA	Ethylenediaminetetraacetic Acid
ELISA	Enzyme-Linked Immunosorbent Assay
IgG	Immunoglobulin G
	National Laboratory for Viral Hemorrhagic
NLVHF	Fevers

NCP	Nucleocapsid Protein
OD	Optical Density
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
WHO	World Health Organization

Conflicts of Interest

The authors declare no conflicts of interest.

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