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Demographic, Seasonal, and Temporal Determinants of SARS-CoV-2 Infection and Serological Status in Al-Bayda City, Libya: A Cross-Sectional Study Employing Multinomial Logistic Regression

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ABSTRACT

This cross-sectional study examined the demographic, seasonal, and temporal determinants of SARS-CoV-2 infection and serological status among individuals tested in Al-Bayda city, eastern Libya, between 2021 and 2025. Despite the global transition toward endemic circulation of SARS-CoV-2, region-specific evidence from North Africa remains limited, particularly with respect to multi-year serological patterns. By applying a multinomial outcome framework, this study aimed to provide a nuanced characterization of population-level exposure beyond binary infection classifications. Laboratory-based serological records were obtained from the Al-Bayda Specialized Diagnostic Laboratory. Individuals were classified into four mutually exclusive serological categories—no exposure, early infection, active infection, and past infection—based on combined IgM and IgG antibody results. Descriptive analyses summarized demographic characteristics and serological distributions, while age differences across outcome categories were evaluated using analysis of variance with post hoc testing. Seasonal and calendar-year patterns were assessed to capture temporal heterogeneity in testing and exposure. To evaluate independent associations of age, gender, season, and calendar year with serological outcomes, multinomial logistic regression with LASSO regularization was employed. Penalized modeling was used to address outcome imbalance, sparse event counts, and potential multicollinearity. Model tuning was conducted using cross-validation, and a conservative regularization parameter was selected to prioritize model stability and generalizability. A total of 309 individuals were included, with a mean age of 32.5 years and an approximately equal gender distribution. Past infection was the most prevalent serological outcome, whereas active and early infections were rare. Unadjusted analyses demonstrated significant age-related differences across serological categories, with older individuals more frequently classified as having active or past infection. However, after adjustment and penalization, none of the examined predictors showed strong independent associations with serological outcomes, as relative risk ratios were approximately unity. These findings suggest that SARS-CoV-2 serological status in this population reflects cumulative and multifactorial exposure processes rather than being driven by isolated demographic, seasonal, or temporal determinants. The use of multinomial ridge regression provides robust region-specific evidence and highlights the importance of advanced analytical approaches in interpreting serological data from resource-limited settings.

Introduction

Since its emergence in late 2019, coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has represented an unprecedented global public health challenge. Although large-scale vaccination campaigns and accumulated population immunity have altered the clinical and epidemiological landscape of COVID-19, the virus continues to circulate worldwide with substantial heterogeneity across demographic groups, seasons, and calendar years. Understanding the determinants of SARS-CoV-2 infection and serological status, therefore, remains essential for interpreting population-level exposure, monitoring immunity dynamics, and informing long-term public health strategies.

Age has consistently emerged as a central determinant in COVID-19 epidemiology. Large meta-analyses and systematic reviews have demonstrated that increasing age is strongly associated with adverse clinical outcomes, including severe disease and mortality [1,2]. Beyond clinical severity, age also influences immune responses following SARS-CoV-2 exposure, affecting both the magnitude and persistence of antibody responses over time. Recent longitudinal and serological studies suggest that age-related immune heterogeneity contributes to distinct patterns of antibody prevalence and durability at the population level [3, 4].

Gender differences in COVID-19 susceptibility, severity, and immune response have also been widely reported. Biological mechanisms, including sex-specific immune regulation, hormonal influences, and differential expression of immune-related genes, may partially explain observed disparities between males

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and females [4]. In parallel, gendered social and economic factors—such as occupational exposure, caregiving roles, and access to healthcare—have shaped differential risks during the pandemic [5,6]. However, findings regarding gender differences in SARS-CoV-2 infection prevalence and serological status remain inconsistent across regions and study designs, highlighting the importance of context-specific investigations.

Seasonality represents another key dimension in the transmission dynamics of SARS-CoV-2. Environmental conditions, population mobility, indoor crowding, and viral stability have all been proposed as contributors to seasonal fluctuations in COVID-19 incidence. Multiple studies have reported higher transmission rates during colder months, although seasonal effects appear to vary across geographical regions and time periods [7,8]. Despite these observations, the influence of seasonality on serological patterns—particularly in terms of cumulative exposure and antibody prevalence—remains incompletely understood.

Serological testing provides critical insight into the full spectrum of SARS-CoV-2 exposure, capturing both symptomatic and asymptomatic infections that may not be identified through molecular testing alone. Immunoglobulin M (IgM) antibodies are generally associated with recent or early infection, whereas immunoglobulin G (IgG) antibodies reflect prior exposure and longer-term immune memory [9,10]. Large-scale seroprevalence studies have demonstrated substantial variability in antibody prevalence across populations and over time, influenced by infection waves, vaccination coverage, and demographic structure [11]. Nevertheless, serological interpretation remains complex due to assay performance variability, timing of sample collection, and the potential for false-positive or false-negative results [12,13].

Despite the expanding literature on COVID-19 epidemiology, many studies rely on simplified binary outcome measures, such as infected versus non-infected, which may obscure important distinctions between active infection, early infection, past infection, and absence of detectable exposure. Multinomial outcome frameworks offer a more nuanced approach by allowing simultaneous evaluation of multiple infection and serological categories. Multinomial logistic regression, in particular, enables robust assessment of demographic, seasonal, and temporal predictors while accounting for potential confounding factors and complex outcome structures.

Importantly, evidence from North African and Libyan contexts remains limited, especially with respect to multi-year serological patterns and their demographic and seasonal determinants. Libya has experienced prolonged health system challenges over the past decade, which may influence testing access, surveillance continuity, and population exposure dynamics. As a result, findings derived from high-income or highly resourced settings may not be directly generalizable to the Libyan population.

Al-Bayda city is a major urban center in eastern Libya and serves a diverse population with varied healthcare-seeking behaviors. Laboratory-based serological data collected over extended periods can therefore provide valuable insights into SARS-CoV-2 exposure patterns within this setting. However, to date, few studies have examined infection status and antibody responses in Libyan populations using advanced multivariable analytical approaches across multiple calendar years.

Accordingly, the present study aims to investigate the demographic, seasonal, and temporal determinants of SARS-CoV-2 infection and serological status among individuals tested at the Al-Bayda Specialized Diagnostic Laboratory in Al-Bayda city, Libya, using cross-sectional data collected between 2021 and 2025. By applying multinomial logistic regression modeling, this study seeks to characterize patterns of active infection, early infection, past infection, and no detectable exposure, thereby contributing region-specific evidence to the broader understanding of SARS-CoV-2 epidemiology and population immunity.

Materials and Methods

Study Design and Setting

This cross-sectional analytical study was conducted using laboratory-based data obtained from the Al-Bayda Specialized Diagnostic Laboratory, a major diagnostic facility located in Al-Bayda city, eastern Libya. The laboratory provides routine and specialized diagnostic services to a diverse urban population and serves as an important referral center in the region. Data were collected over a prolonged period from January 2021 through December 2025, allowing assessment of both seasonal and temporal trends in SARS-CoV-2 infection and serological status.

Study Population and Data Collection

The study population comprised individuals who underwent serological testing for SARS-CoV-2 antibodies during the study period. Laboratory records were reviewed retrospectively. Only records containing complete information on age, gender, date of testing, season of sample collection, and IgG and IgM antibody results were included in the analysis. Records with missing, inconclusive, or invalid serological results were excluded to ensure data integrity.

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Serological Testing and Interpretation

Serological testing was performed using a COVID-19 Antibody Rapid Test designed for the qualitative detection of IgM and IgG antibodies against SARS-CoV-2 in human blood samples. All assays were conducted in accordance with the manufacturer's instructions as part of routine diagnostic procedures.

IgM antibodies were interpreted as markers of recent or early immune response, whereas IgG antibodies were considered indicative of prior exposure and longer-term immune memory, consistent with established immunological principles in SARS-CoV-2 infection [9,10]. Based on the combined IgM and IgG results, individuals were classified into one of four mutually exclusive categories: early infection, active or recent infection, past infection, or no serological evidence of exposure. This classification approach allowed differentiation between recent, ongoing, resolved, and undetectable exposure states.

The potential limitations of rapid serological testing were recognized, particularly the influence of timing of sample collection on antibody detectability and the possibility of false-positive or false-negative results, as reported in previous evaluations of IgG/IgM assays (12).

Variables

The primary outcome variable was SARS-CoV-2 infection and serological status, categorized into four outcome groups as described above. Independent variables included age (measured in years), gender (male or female), season of sample collection (winter, spring, summer, or fall), and calendar year of testing (2021–2025).

Statistical Analysis

All statistical analyses were conducted using R software version 4.4.2. Data management, cleaning, and analysis were performed using the following R packages: readr, tidyverse, dplyr, janitor, scales, ggplot2, tableone, epitools, broom, and glmnet.

Descriptive statistics were used to summarize demographic characteristics and serological outcomes. Continuous variables were expressed as means with standard deviations, while categorical variables were reported as frequencies and percentages. Associations between categorical variables were assessed using Fisher's exact test or Pearson's chi-square test, as appropriate. Differences in mean age across serological outcome groups were evaluated using analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons.

To identify independent demographic, seasonal, and temporal predictors of SARS-CoV-2 infection and serological status, a multinomial logistic regression model was fitted, with the *no exposure* category specified as the reference outcome. Penalized multinomial regression with least absolute shrinkage and selection operator (LASSO) regularization was applied to minimize overfitting and stabilize coefficient estimates in the presence of multiple covariates. Model tuning parameters were selected using cross-validation procedures. Regression results were expressed as relative risk ratios (RRRs) derived from exponentiated model coefficients. All statistical tests were two-sided, and a p-value of less than 0.05 was considered statistically significant.

Ethical Considerations

The study utilized anonymized secondary laboratory data collected as part of routine diagnostic practice. No personal identifiers were accessed or retained. The study involved minimal risk to participants and was conducted in accordance with ethical principles governing the use of secondary health data.

Results

Descriptive Characteristics of the Study Population

A total of 309 individuals were included in the final analysis. The overall mean age of the participants was 32.52 years (SD = 27.80), indicating a wide age distribution encompassing children, young adults, and older individuals. The gender distribution was nearly balanced, with 155 females (50.2%) and 154 males (49.8%), suggesting minimal gender-based sampling bias.

Regarding seasonality, the majority of samples were collected during fall (38.8%) and winter (35.6%), while fewer samples were obtained during summer (12.9%) and spring (12.6%). This distribution reflects higher testing activity during cooler seasons. In terms of calendar year, the largest proportion of samples was collected in 2022 (37.5%), followed by 2023 (23.6%) and 2024 (19.1%). Smaller proportions were recorded in 2021 (11.0%) and 2025 (8.7%), reflecting variability in testing demand and surveillance intensity over time. Based on combined IgG and IgM antibody results, past infection was the most prevalent serological outcome, accounting for 169 individuals (54.7%). No serological evidence of exposure was identified in 129 individuals (41.7%). In contrast, active infection (1.9%) and early infection (1.6%) were relatively rare.

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Table 1. Descriptive statistics of the study population (N = 309)

Variable	Category	n (%) / Mean (SD)
Age	—	32.52 (27.80)
Gender	Female	155 (50.2 %)
	Male	154 (49.8 %)
Season	Winter	110 (35.6%)
	Spring	39 (12.6 %)
	Summer	40 (12.9 %)
	Fall	120 (38.8 %)
Year	2021	34 (11.0 %)
	2022	116 (37.5 %)
	2023	73 (23.6 %)
	2024	59 (19.1 %)
	2025	27 (8.7 %)
Results	Active Infection	6 (1.9 %)
	Early Infection	5 (1.6 %)
	No Exposure	129 (41.7 %)
	Past Infection	169 (54.7 %)

Distribution of SARS-CoV-2 Serological Status by Gender

The distribution of SARS-CoV-2 serological outcomes by gender is presented in (Figure 1). In both females and males, past infection represented the dominant category, followed by no exposure. Active infection was observed exclusively among females, whereas early infection was slightly more frequent among males. Despite these numerical differences, the overall distribution of serological outcomes appeared broadly comparable between genders.

Seasonal Distribution of SARS-CoV-2 Serological Outcomes

(Figure 2) illustrates the distribution of serological outcomes across seasons. Past infection remained the most frequent outcome in all seasons, with the highest absolute counts observed during fall and winter, corresponding to the periods with the greatest testing volume. No exposure was also consistently observed throughout the year. Active and early infections were infrequent and did not display clear seasonal clustering.

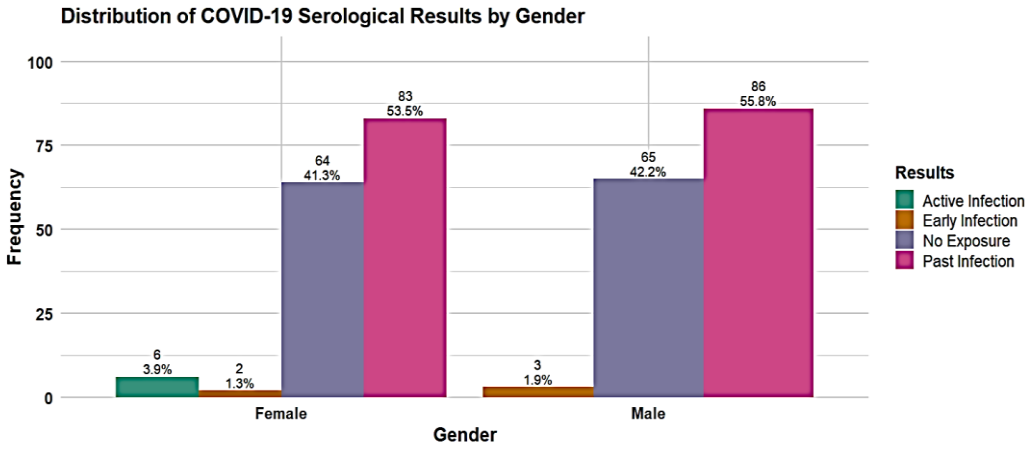

Figure 1. Grouped bar chart showing the distribution of SARS-CoV-2 infection and serological status by gender. Past infection is the most prevalent outcome in both females and males, while active and early infections are rare.

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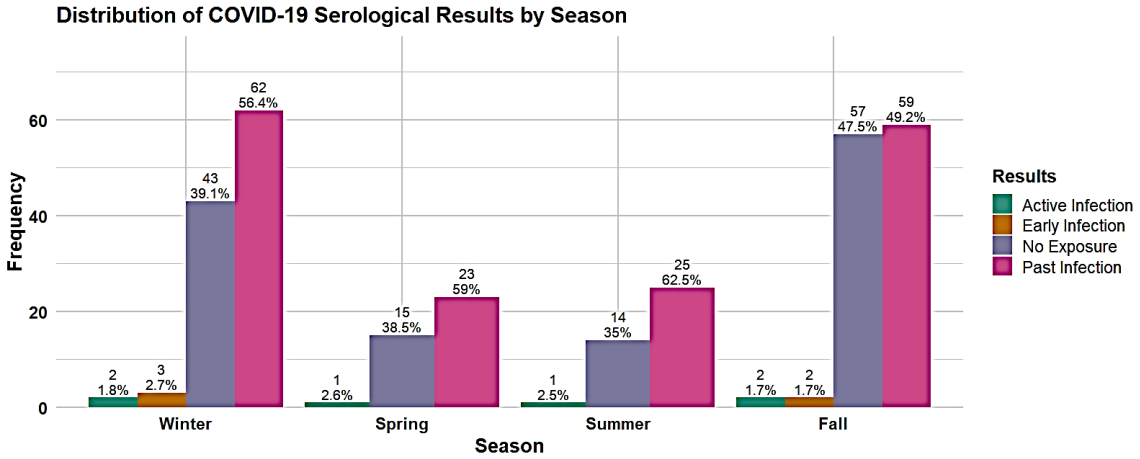

Figure 2. Grouped bar chart showing the distribution of SARS-CoV-2 infection and serological status by season of testing. Past infection predominates across all seasons, with higher absolute frequencies observed during fall and winter.

Temporal Distribution Across Calendar Years

The distribution of serological outcomes by calendar year is shown in (Figure 3). Past infection predominated in all years, with the highest number recorded in 2022, coinciding with the peak testing period. The proportion of individuals with no serological evidence of exposure remained relatively stable across years. Active and early infections were sporadically observed and represented a small fraction of cases in each year.

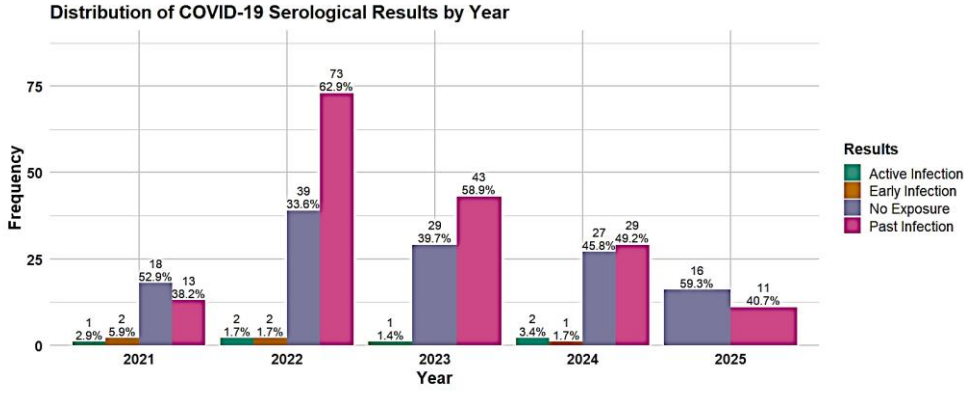

Figure 3. Grouped bar chart showing the distribution of SARS-CoV-2 infection and serological status by calendar year (2021–2025). Past infection remains the dominant category throughout the study period, with peak frequencies in 2022.

Age Distribution Across SARS-CoV-2 Serological Outcomes

Age differed significantly across SARS-CoV-2 serological outcome categories. The mean age was highest among individuals classified with active infection (mean = 59.3 years, SD = 25.8), followed by those with past infection (mean = 36.6 years, SD = 28.3). In contrast, participants categorized as having no exposure had a lower mean age (26.4 years, SD = 26.0), while the early infection group exhibited the youngest age profile (mean = 18.8 years, SD = 16.8).

One-way analysis of variance (ANOVA) demonstrated a statistically significant overall difference in age across serological categories ($F = 5.83$, $p < 0.001$), indicating that age distribution varied meaningfully by infection or exposure status. Post hoc pairwise comparisons using Tukey's method revealed that individuals with no exposure were significantly younger than those with active infection (mean difference = −32.9 years, $p = 0.021$). Additionally, individuals with past infection were significantly older than those with no exposure (mean difference = 10.2 years, $p = 0.008$).

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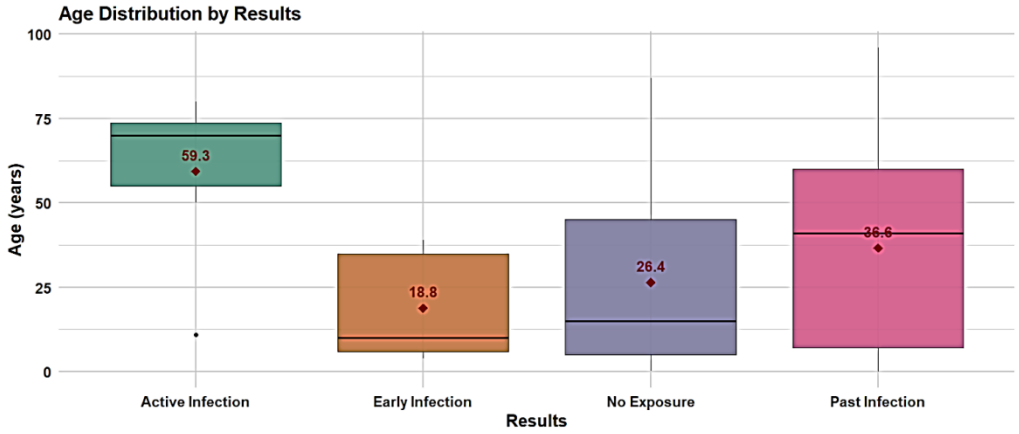

Figure 4. Boxplot showing age distribution across SARS-CoV-2 serological outcome categories. Median age, interquartile range, and extreme values are displayed. Significant differences in age distribution across outcomes were observed.

Other pairwise comparisons did not reach statistical significance, although a borderline difference was observed between early infection and active infection (p = 0.068), likely reflecting limited sample size in these categories. The boxplot representation (Figure 4) visually corroborates these findings, showing broader age dispersion among past infection cases and a right-shifted age distribution for active infection.

Model Tuning and Cross-Validation of Multinomial LASSO Regression

To evaluate the joint effects of age, gender, season, and calendar year on SARS-CoV-2 serological outcomes, a multinomial logistic regression model was fitted with No Exposure as the reference category. Given the marked imbalance between outcome groups and the possibility of multicollinearity among predictors, LASSO regularization was applied to enhance model stability and prevent overfitting.

Model tuning was performed using k-fold cross-validation. The optimal penalty parameter (λ) was selected based on the minimum cross-validated multinomial deviance, while a more conservative λ_1se value was retained in the final model to prioritize parsimony and robustness. At the selected penalty level, coefficient shrinkage substantially attenuated the effects of all predictors. Exponentiated coefficients (RRRs) were close to unity across outcome categories, indicating the absence of strong independent associations after penalization.

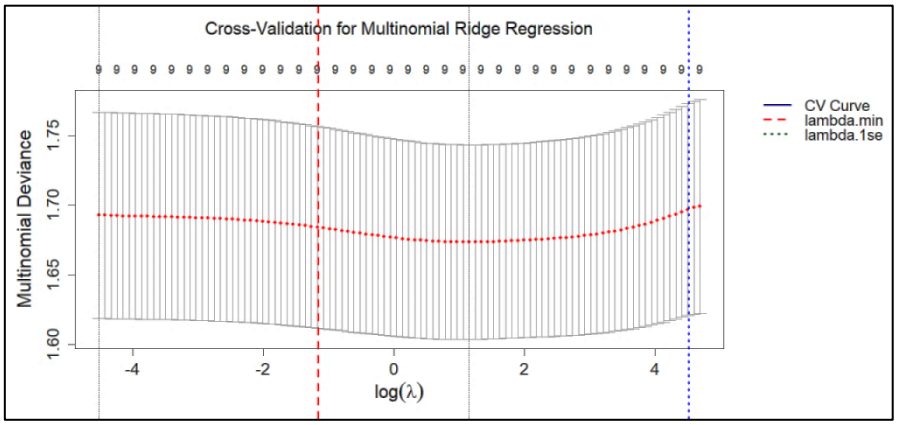

Figure 5. Cross-validation curve for multinomial ridge regression showing the relationship between log(λ) and cross-validated multinomial deviance. The selected λ_1se represents a conservative penalty optimizing model stability and generalizability.

At the selected λ_1se, coefficient shrinkage substantially attenuated the effects of all predictors across outcome categories. Exponentiated coefficients, expressed as relative risk ratios (RRRs), were approximately unity for age, gender, season, and calendar year when comparing active infection, early infection, and past infection with the reference group. This indicates that no predictor demonstrated a strong independent association with serological status after adjustment and penalization.

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Although age exhibited statistically significant differences across serological outcome groups in unadjusted analyses, it did not retain an independent association in the adjusted multinomial model. This attenuation suggests that the observed age-related patterns were partially explained by overlapping seasonal and temporal effects, shared variance with other covariates, or outcome group imbalance rather than representing a robust standalone determinant. Similarly, gender did not show differential adjusted risks across serological outcomes, despite minor descriptive differences observed in univariate distributions.
Season of sample collection and calendar year also failed to demonstrate independent effects on serological classification after regularization, indicating that temporal and seasonal variations observed descriptively did not translate into stable multivariable associations. In contrast, intercept terms differed across outcome categories, reflecting baseline differences in outcome prevalence within the study population.
Overall, the multinomial ridge regression model emphasized robustness and predictive stability over effect magnitude estimation. The findings suggest that SARS-CoV-2 serological status in this population reflects a multifactorial process rather than being driven by isolated demographic, seasonal, or temporal predictors when considered jointly under conservative regularization.

Discussion

This study provides a comprehensive assessment of demographic, seasonal, and temporal determinants of SARS-CoV-2 infection and serological status in Al-Bayda city, Libya, using a multinomial outcome framework across five years (2021–2025). By distinguishing between active infection, early infection, past infection, and no detectable exposure, the analysis offers a nuanced understanding of population-level exposure patterns that extends beyond binary infection classifications commonly used in the literature.

Age-Related Patterns and Serological Outcomes

Age emerged as a key factor in unadjusted analyses, with significant differences observed across serological outcome categories. Individuals with active infection were notably older on average than those classified as having no exposure, while past infection was also associated with higher mean age. These findings are consistent with global evidence demonstrating age-related heterogeneity in SARS-CoV-2 exposure and immune response dynamics [1,2,3]. Older individuals may accumulate greater exposure risk over time due to repeated contacts, comorbidities requiring healthcare visits, or reduced immune efficiency, leading to more detectable antibody responses.
However, the attenuation of age effects in the adjusted multinomial ridge regression model suggests that age alone does not independently determine serological classification when seasonal and temporal factors are considered simultaneously. This finding highlights the importance of accounting for correlated predictors and outcome imbalance when interpreting age-associated patterns. It also suggests that observed age differences may reflect cumulative exposure shaped by broader epidemiological dynamics rather than intrinsic age-specific susceptibility alone.

Seasonality and Temporal Dynamics

Seasonal variation in testing volume and serological outcomes was evident descriptively, with the highest number of samples collected during fall and winter. This pattern mirrors established seasonal trends in SARS-CoV-2 transmission reported in diverse geographical settings, where colder temperatures, increased indoor crowding, and reduced ventilation facilitate viral spread [7,8]. Nevertheless, active and early infections were infrequent across all seasons, and seasonal variables did not retain independent associations in the adjusted model.
The lack of strong independent seasonal effects after penalization suggests that seasonality may influence exposure risk indirectly—through behavioral and testing patterns—rather than exerting a dominant effect on serological classification at the individual level. Similarly, calendar year did not independently predict serological outcomes in the adjusted analysis, despite clear variations in testing intensity across years. These findings underscore the complexity of disentangling true epidemiological trends from surveillance artifacts, particularly in settings with fluctuating testing practices.

Frequent Previous Infections and High Prevalence of Past Exposure

The predominance of past infection observed in this study, accounting for more than half of all participants, reflects substantial cumulative exposure to SARS-CoV-2 within the study population. This finding aligns with global seroprevalence estimates indicating widespread exposure following multiple pandemic waves, particularly after the emergence of highly transmissible variants [11]. The high prevalence of IgG-positive individuals suggests repeated or prior infections rather than sustained transmission of active disease at the time of testing.

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Importantly, the low proportion of active and early infections likely reflects the timing of serological testing relative to infection, as well as the inherent limitations of rapid antibody assays in detecting very recent infections (12,13). These findings support the interpretation that serological surveillance primarily captures historical exposure rather than real-time transmission dynamics, reinforcing the value of combining serology with molecular testing in comprehensive surveillance strategies.

Gender Differences in Context

Despite biological and social mechanisms proposed to underlie gender differences in COVID-19 susceptibility and outcomes [4, 5], this study did not identify big gender-based differences in serological outcomes after adjustment. While minor descriptive differences were observed—such as active infections occurring exclusively among females—these did not persist in the multivariable model. This result aligns with several seroprevalence studies reporting inconsistent or context-dependent gender effects and suggests that gender-related disparities may be more pronounced for disease severity than for cumulative exposure or antibody prevalence.

The Libyan Context and Public Health Implications

The findings must be interpreted within the broader Libyan context, characterized by prolonged health system constraints, variable testing access, and intermittent surveillance capacity. These factors likely influence who seeks testing, when testing occurs, and which infections are ultimately captured in laboratory records. Consequently, the absence of strong independent predictors in the adjusted model may reflect heterogeneous exposure pathways and incomplete surveillance rather than a true absence of demographic or seasonal effects.

Nonetheless, the study provides valuable region-specific evidence from eastern Libya, addressing a notable gap in the literature. The use of multinomial ridge regression allowed robust modeling despite outcome imbalance and limited event counts, prioritizing stability and generalizability over potentially spurious effect estimates. This approach is particularly appropriate in resource-limited settings where data sparsity is common.

Integration with Global Evidence

Overall, the findings support the interpretation that SARS-CoV-2 serological status in this population was shaped by a complex interaction of demographic and temporal factors rather than by any single dominant predictor. The attenuation of crude associations once penalized multivariable modeling was applied underscores the importance of using analytical approaches capable of addressing multicollinearity and outcome imbalance in epidemiological datasets [14,15,16,17]. This approach enhances the robustness of inference and reduces the risk of over-interpreting spurious associations.

Strengths and Limitations

Key strengths of this study include the multi-year timeframe, the use of multinomial outcome classification, and the application of penalized regression to address imbalance and multicollinearity. However, limitations include the cross-sectional design, reliance on rapid serological assays, absence of vaccination status data, and potential selection bias related to healthcare-seeking behavior. These factors may limit causal inference and generalizability beyond the study population.

Taken together, these findings indicate that serological status in this population reflects cumulative exposure processes operating across multiple demographic and temporal contexts, rather than being driven by isolated individual-level predictors. The attenuation of effects in the penalized multivariable model highlights the importance of using conservative analytical approaches when working with imbalanced serological outcomes in resource-limited surveillance settings. Future research should incorporate vaccination history, molecular testing data, and larger multi-site samples to better disentangle behavioral, immunological, and structural determinants of SARS-CoV-2 exposure patterns in Libya and comparable contexts.

Conclusions

In conclusion, this study demonstrates that SARS-CoV-2 serological outcomes in Al-Bayda City are shaped by cumulative and multifactorial exposure dynamics rather than by single demographic, seasonal, or calendar-year determinants. While age-related differences were evident in descriptive analyses, these effects diminished after adjustment and penalization, and neither gender, season, nor year retained independent predictive value in the final model. These findings underscore the importance of context-specific sero-

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epidemiological analyses using robust statistical frameworks and contribute novel regional evidence from Libya to the broader understanding of post-pandemic population immunity.

Conflict of interest. Nil

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