Convergence of SARS-CoV-2 spike antibody levels to a population immune setpoint

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Summary

Background Individual immune responses to SARS-CoV-2 are well-studied, while the combined effect of these responses on population-level immune dynamics remains poorly understood. Given the key role of population immunity on pathogen transmission, delineation of the factors that drive population immune evolution has critical public health implications.

Methods We enrolled individuals 5 years and older selected using a multistage cluster survey approach in the Northwest and Southeast of the Dominican Republic. Paired blood samples were collected mid-pandemic (Aug 2021) and late pandemic (Nov 2022). We measured serum pan-immunoglobulin antibodies against the SARS-CoV-2 spike protein. Generalized Additive Models (GAMs) and random forest models were used to analyze the relationship between changes in antibody levels and various predictor variables. Principal component analysis and partial dependence plots further explored the relationships between predictors and antibody changes.

Findings We found a transformation in the distribution of antibody levels from an irregular to a normalized single peak Gaussian distribution that was driven by titre-dependent boosting. This led to the convergence of antibody levels around a common immune setpoint, irrespective of baseline titres and vaccination profile.

Interpretation Our results suggest that titre-dependent kinetics driven by widespread transmission direct the evolution of population immunity in a consistent manner. These findings have implications for targeted vaccination strategies and improved modeling of future transmission, providing a preliminary blueprint for understanding population immune dynamics that could guide public health and vaccine policy for SARS-CoV-2 and potentially other pathogens.

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Research in context

Evidence before this study

The level of immune protection across a population strongly influences the magnitude and severity of infectious disease epidemics. However, understanding how individual-level factors such as infection, vaccination, immune waning, or host immunogenicity influence population immunity and the evolution of population has not been well characterized. We searched PubMed from January 2020 to April 2024 with the following search terms: "SARS-CoV-2" AND "population immunity" AND "antibody response" AND "immune dynamics" OR "Immunoepidemiology" Studies were included if they provided data on population-level immune responses to SARS-CoV-2, measured antibody levels, and were conducted in diverse geographic regions. We found limited relevant literature with most population-level studies focusing on cross-sectional seroepidemiological studies of risk factors for infection, transmission dynamics, and epidemiological parameters. Some cohort studies examined antibody kinetics over time among individuals infected, vaccinated, or both, with findings suggesting that the magnitude of immune boosting declines following multiple antigen exposures. Some studies examined the role of population immunity on viral evolution, post-acute sequelae of COVID-19, or both. However, no studies explicitly examined how the distribution of antibody levels across a population evolve over time or explore the concept of environmental/viral pressure driving population to an immune setpoint.

Added value of this study

This study provides insights into the evolution of population immunity to SARS-CoV-2. Our research examines how the distribution of antibody levels evolved across a population in a high transmission setting, from mid pandemic to late pandemic. We identify a consistent pattern of immune marker convergence towards a single-peak Gaussian distribution, driven by titre-dependent boosting, which has not been previously documented. These findings highlight the concept of a population immune setpoint, suggesting that widespread transmission and vaccination drive immune responses to similar levels across a population. This understanding has implications for tailoring vaccination strategies, enhancing epidemiological modeling, and informing public health policies to effectively manage SARS-CoV-2 and other epidemic pathogens.

Implications of all the available evidence

Our findings build on an expansive knowledge base of SARS-CoV-2 epidemiology and individual-level immunity to discern how humoral immunity evolves among populations exposed to serial waves of transmission.

Introduction

Population-level immunity is arguably the most important predictor of transmission dynamics for infectious pathogens. Despite this, the investigation of population immunity and immune dynamics, known as immunoepidemiology, remains largely overshadowed by the focus on individual immune responses. While the study of population immune dynamics is relatively simple for certain pathogens such as measles that generate complete, or near complete, and long-term immune protection following infection or a primary vaccine series, it is substantially more difficult for SARS-CoV-2 and other epidemic-prone pathogens that generate partial and transient immune protection.[1] Given this, only a small number of studies have aimed to characterize population immune protection during the pandemic,[2–4] and these studies have focused on cross-sectional estimates with their public health utility limited by rapid changes in population immunity and emergence of more immune-evasive variants.[5] The question of how population immunity evolves over time and whether there are generalizable principles that underpin this evolution is unexplored.

The humoral response following antigen exposure is dynamic, typically characterized by immunological boosting and high levels of protection in the several months after vaccination or infection. Antibody levels then decline over time, with a rapid drop in the first 6–12 months followed by a slower decline or stabilization thereafter.[6–8] Yet, despite these overall trends, individual immune responses are highly variable, influenced by the number and timing of vaccine doses or infections, severity of infection, and individual differences in the host immune response. As such, and when considered in the context of declining vaccination uptake and widespread undetected and/or unreported infections, understanding population immunity and immune markers dynamics is a considerable challenge.

Given these challenges, we aimed to understand if patterns or trends in antibody dynamics assessed on a population level may provide insights into the factors that drive the evolution of population immunity. Following the introduction of a novel pathogen into a wholly susceptible population, particularly one that generates partial and transient immune protection such as SARS-CoV-2, the distribution of population immunity and immune marker levels early in the pandemic would, in most scenarios, be highly irregular. This was demonstrated across multiple population-based studies early in the COVID-19 pandemic.[3,5,9,10] However, whether this irregular distribution of antibody levels would persist or whether the combined influence of vaccinations, infections and humoral waning would lead

to alternate patterns is largely unknown. Data from a single prior study that measured SARS-CoV-2 spike-antibody levels among patients enrolled across an acute febrile infection surveillance platform in the Dominican Republic suggests that population immunity may not remain disordered but rather converge towards a predictable pattern, with normalization in the distribution of antibody levels from an irregular to a single Gaussian peak over time.[5] But these findings have not been replicated and the design of the study precluded analysis of factors driving the observed changes.

To investigate this potential convergence phenomenon and the factors that drive the evolution of population immune markers we analyzed serological data from a cohort study in the Dominican Republic. By examining the dynamics of population immune markers, we aimed to understand how and why population immunity evolves—with the ultimate goal of informing vaccination and public health policy.

Methods

Settings, COVID-19 vaccine characteristics and national control measures

The Dominican Republic is an upper middle income Latin American country that shares the island of Hispaniola with Haiti. With almost 11 million residents, it is the second most populous country in the Caribbean.[11,12] The first laboratory confirmed case of SARS-CoV-2 was reported in the Dominican Republic on 1 March 2020, and strict public health measures commensurate with most regional countries were implemented. Six discrete waves of transmission occurred between March 2020 and December 2022 (Fig. 1A). The Dominican Republic launched a national COVID-19 vaccination campaign in February 2021, and in July 2021 was the first country in the Americas to authorize third doses for high-risk individuals. Vaccination coverage increased rapidly from Mar to Nov 2021 but slowed thereafter (Fig. 1B). The principal COVID-19 vaccines administered included the inactivated viral CoronaVac (Sinovac), that generates antibodies to the spike and nucleocapsid proteins, and the adenovirus vector ChAdOx1-S (Oxford/AstraZeneca) and mRNA BNT162b2 (Pfizer/BioNTech) vaccines, which generate only anti-spike antibodies. On Feb 16, 2022, the Dominican Republic lifted all COVID-19-related requirements and restrictions including mask mandates, proof of vaccination to enter venues, and business capacity limitations. Based on a national household serological survey conducted between 30 June and 12 October 2021, which provides the first of the two sampling timepoints for the present study and conducted between the third (Mu) and fourth (Delta) waves of transmission (Fig. 1A), 77.6% (CI 71.1–83.4) of the population aged ≥ 5 years were estimated to have been previously infected at least once.[3] Overall, 37.5% (CI 32.9–41.3) of the ≥5-year-old population were estimated to have been previously infected and vaccinated, 40.1% (CI 38.2–42.0) previously infected but not vaccinated, 11.9% (CI 8.1–16.5) vaccinated but not infected, and 10.5% (CI 8.6–12.3) neither vaccinated nor infected.[3]

Study design, participant selection, and ethical considerations

As previously described,[3] between June 30 and Oct 10, 2021, we conducted a three-stage cross-sectional national household serological survey and enrolled 6683 participants from 3832 households, and 134 clusters across all 32 provinces nationally. Briefly, to maximize spatial distribution we used grid- and satellite-image based methods to select clusters and 60 households within each cluster.[13] Household members aged ≥ 5 years old present in the home at the time of the serosurvey were invited to participate. During October and November 2022, we conducted repeat blood sampling across 38 of the 134 clusters in four provinces in the northwest and southeast of the country, defined for analysis purposes as Northwest Region and Southeast Region. Clusters were balanced between urban and rural and selected given proximity to existing longitudinal acute febrile infection surveillance sites in Espaillat and San Pedro de Macoris provinces, operated by the study team in partnership with the Ministry of Health and Social Assistance. Written consent was obtained for all participants and provided by (i) the participating adult for individuals ≥18 years, (ii) the legal guardian for non-emancipated minors, and (iii) the participating emancipated minor where applicable. Emancipated minors were defined as individuals less than 18 years of age that are either married, pregnant, a parent, living independently of parents, or legally emancipated. In addition to written consent, written assent was provided by adolescents 14–17 years old, and verbal assent by children 7–13 years old. The study protocol was approved by the National Council of Bioethics in Health, Santo Domingo (013-2019, 007-2021), the Institutional Review Board of Pedro Henríquez Ureña National University, Santo Domingo, and the Mass General Brigham Human Research Committee, Boston, USA (2019P000094, 2021P001294). All participants enrolled in the 38 prespecified clusters were invited to participate in resampling and therefore no sample size calculation was performed. Study procedures and reporting adhered to STROBE criteria for observational studies.

Study procedures

Questionnaires were administered to all study participants using the KoBo Toolbox data collection platform (www.kobotoolbox.org) on electronic tablets to collect self-reported individual-level covariates including demographics (age, gender, race-ethnicity); comorbid medical conditions; weight and height; primary occupation; if the work location was primarily indoors,

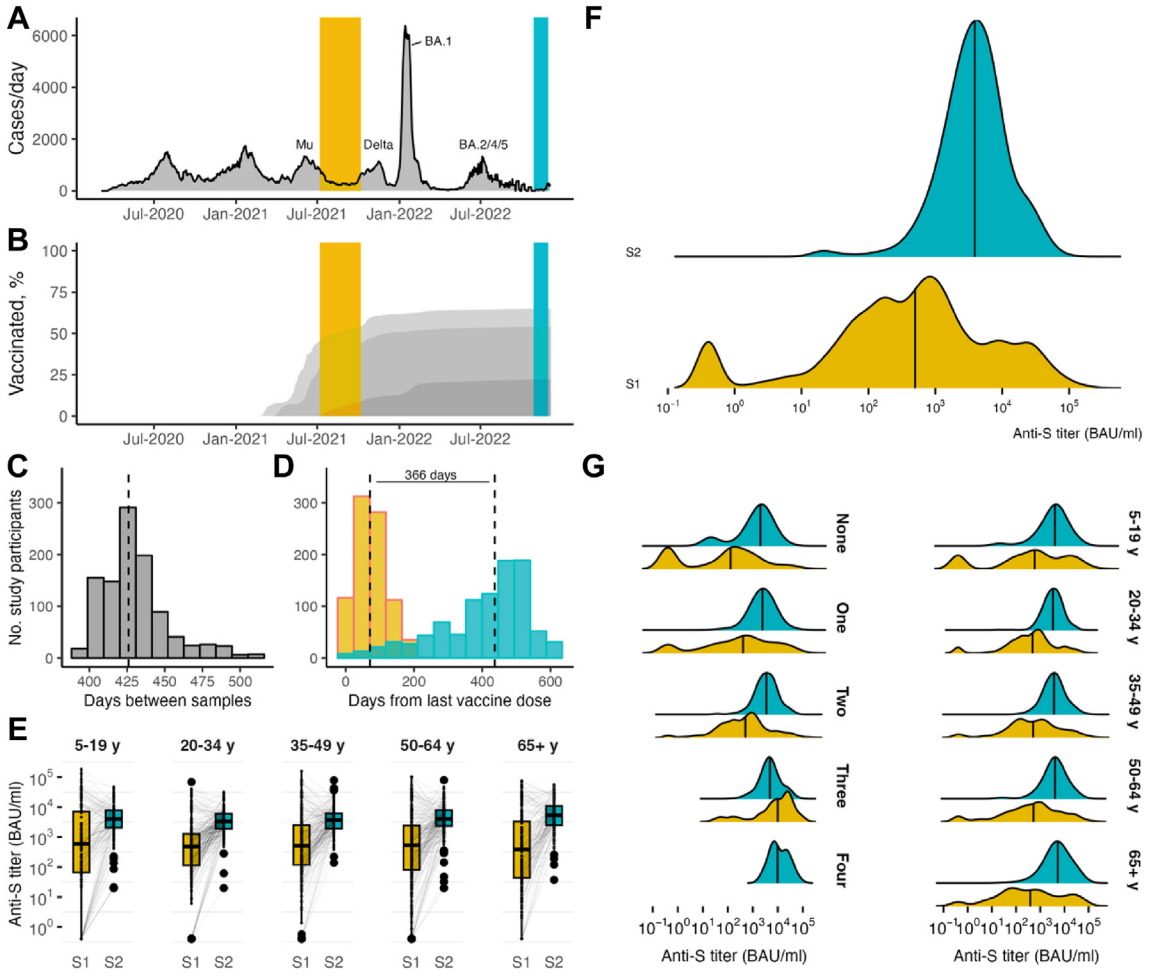

Fig. 1: **Individual and population changes in SARS-CoV-2 spike antibody levels. (A)** National reported SARS-CoV-2 cases per day with predominant circulating variant denoted. Limited sequencing data is available prior to mid-2021. Vertical shading indicates timing of the sampling periods: 5 Jul to 10 Oct 2021 (S1, yellow) and 29 Oct to 29 Nov 2022 (S2, green). **(B)** Nationally reported COVID-19 vaccine coverage with dark gray indicating three or more doses, gray two or more doses, and light gray one dose. Data on national SARS-CoV-2 cases and COVID-19 vaccinations used in plot A and B are available from https://ourworldindata.org/coronavirus. Data on predominant circulation viral variants were previously reported.[10] **(C)** Histogram plot of the number of days between paired sample collection with vertical dashed line indicating the median value (426 days). **(D)** Histogram plot of the number of days from the last vaccine dose at the first sampling timepoint (yellow, S1) and the second sampling timepoint (green, S2). Vertical dashed lines indicate median values (71 and 437 days, respectively). Underlying data are summarized in Supplementary Tables S1 and S2. **(E)** Boxplots show the spike antibody levels across the two sampling periods stratified by age group. The thick horizontal line indicates the median value, with lower and upper hinges representing the first and third quartiles. Whiskers indicate values between the first quartile and the lowest value, and third quartile and highest value, respectively, but no further than the 1.5 x the interquartile range. Values outside this range are plotted individually. The thin black lines connecting time periods indicates changes in individual participant spike antibody levels. **(F)** Ridge plots indicates the density of spike antibody titres across the study population at the two sampling time points. **(G)** Ridge plots as in plot F stratified by the total number of vaccine doses received at each sampling time point (left), and age (right). N = 1026 for all plots except A and B that use national reported data.

outdoors, or a mix of the two; smoking status; and number, date, and type of COVID-19 vaccine received. Venous blood was collected, processed as sera, and frozen at –80 C.

Immunoassay characteristics

Serum pan-immunoglobulin antibodies against the SARS-CoV-2 spike and nucleocapsid glycoprotein were measured at the Brigham and Women's Hospital, Boston, USA, on the Roche Elecsys SARS-CoV-2 electrochemiluminescence immunoassay that uses a recombinant protein modified double-antigen sandwich format (Roche Diagnostics, Indianapolis, USA). The S assay targets antibodies to the S1 subunit of the spike protein based upon the ancestral Wuhan Hu-1 strain. The assay was calibrated with positive and negative

quality controls before analyses. Values were quantified between 0.40 and 250 U/mL representing the primary measurement range, with values below 0.40 U/mL reported as 0.40 U/mL. Samples with S-antibody values > 250 U/mL underwent automated 1:50 dilution with further 1:10 dilution for samples >12,500 U/mL, representing an upper limit of detection of 125,000 U/mL. Samples were considered reactive according to the manufacturer cutoff index (S-antibody level ≥ 0.8 U/mL). Values are reported as binding antibody units (BAU), that approximate Elecsys SARS-CoV-2 S-antibody U/mL in accordance with manufacturer's recommendations and the WHO International Standard and International Reference Panel for S-antibody SARS-CoV-2 immunoglobulin.[14] Antibodies to the nucleocapsid glycoprotein were only measured on samples from the first sampling timepoint.

Classification and statistical analysis

All analyses and data visualization were performed using the R statistical programming language (R version 4.3.0, 2023-04-21), using *ggplot2* for data visualization.[15]

Regression analyses

We used GAMs to investigate the relationship between change in interval S-antibody levels and various factors. S-antibody change was calculated by subtracting the log10 scale antibody level at the first sampling timepoint from the log10 antibody level at the second sampling timepoint. We fitted GAMs to the data using the *mgcv* package, with the dependent variable being the change in S-antibody levels between the sampling timepoints. The model incorporated predictor variables that influence or may influence exposure risk or differential antibody contraction, including baseline S-antibody and N-antibody levels, age, sex, smoking status, urban versus rural setting, number of COVID-19 vaccine doses prior to the first sampling period (S1), days since last COVID-19 vaccine dose, and days between sample collection. Given that self-reported prior COVID-19 infection has been documented to be unreliable, an assertion supported by our cohort that reported ~10% incidence of prior infection (Supplementary Table S1), this variable was not included in this or subsequent models. We generated plots to visualize the relationship between the baseline S-antibody level and the outcome variable by the number of interval vaccine doses received, given this was a priori the most important variable in determining the change in interval antibody levels.

Model prediction and out-of-sample validation

Our analysis involved three different methods of model training and validation: Regional split, random split, and 10-fold cross-validation. For all models, we used the random forest algorithm implemented in the *randomForest* package. In the first method, we split the data based on the study region. Observations from the Southeast region were used to train the model, and observations from the Northwest region were used to test the model. This method was used to assess the model's ability to generalize across different geographical regions. The random forest model was trained with 500 trees and 3 random features tried at each split. The second method involved randomly shuffling the data and splitting it into training and test sets at a 70:30 ratio. The random forest model was again trained using the same formula as previously. In the third method, we used 10-fold cross-validation using the *caret* package, splitting the data into a training set (70%) and a test set (30%). The training data was further split into 10 folds. For each iteration, the model was trained on 9 folds and validated on the remaining fold. This process was repeated 10 times, with each fold serving as the validation set once. The final model was then evaluated on the unseen test data. We used the mean squared error (MSE), root mean squared error (RMSE), R2 score, and Pearson correlation coefficient as metrics to evaluate model performance.

Principal component analysis

We performed principal component analysis (PCA) to explore the relationships between predictor variables and S-antibody change (Fig. 3A). First, we selected predictor variables based upon variable identified in the prior regression analyses as being consistently associated with interval S-antibody change, including age, log scaled baseline S and N titres, vaccination doses prior to and during the interval, and number of days since the last COVID-19 vaccine dose. We scaled and centered the data prior to performing PCA. To create a biplot of the PCA results, we used the *ggbiplot* function and modified the aesthetics to include groups based on the number of interval vaccine doses.

Partial dependence plots

Partial dependence plots were created to visualize the relationship between the change in S-antibody titre levels between the two sampling time points and various predictor variables. A random forest model with the *randomForest* package was fitted using the following predictor variables: baseline S-antibody level, baseline N-antibody level, age, days post last COVID-19 vaccine dose (DPV), number of interval vaccine doses received between the two time points, number of vaccine doses received prior to the first sampling time point. The random forest model was built using 500 trees, with an *mtry* of 12 selected using a 5-fold cross-validation process. For each predictor variable, a partial dependence plot was generated using the *pdp* package. The partial function was used to calculate the marginal effect of each predictor variable on the change in S-antibody titre levels, holding all other variables constant at their average values. Scatter plots with jittered points were

created to represent individual data points, and post-hoc smoothing was performed using the gam function. p-values were calculated for each predictor variable using GAM models, which were fitted to the change in S-antibody levels and the respective predictor variable. p-values were obtained from the summary of the GAM models, indicating the significance of the term of the predictor variable in explaining the change in S-antibody levels.

Sensitivity analyses

To assess for potential regression towards the mean, a subset of individuals with the top and bottom 5% of S-antibody levels were selected. The baseline S-antibody measurements within this subset were then shuffled randomly using the *sample* function and a linear regression model was fit using shuffled values to predict the actual subsequent measurements. We then examined the impact of extreme baseline S-antibody values on subsequent readings. After identifying and excluding outliers, defined as values falling outside 1.5 times the IQR above the 75th percentile and below the 25th percentile, we subjected the data (both full and without outliers) to three cross-validation methods as previously described: region-based, random split, and 10-fold. For each, a general additive model was used, and performance metrics including MSE, RMSE, R2R2, and correlation were calculated. To assess the influence of outlier removal, differences in metrics between the two datasets were assessed.

Modeling titres as a dynamical system

To further explore principles underlying the evolution of population immune titres we considered various scenarios, including the scenario that population titres converge over time as suggested by prior studies.[5] The converge of titres to a set point may be explained in terms of dynamical system theory: if x is the initial titre, then and the post-infection titre is defined by some function f(x). If $f(x^*) = x^*$ has a non-zero solution x^*, then the fixed point will be stable—i.e., converge over time—if $|f(x^*)|<1$. To visualise these theoretical explanations for convergence, we simulated two distributions of titres. First, we generated a 'low titre distribution', with 100 normally distributed random numbers with mean = 1 and sd = 2. Second, we generated a 'high titre distribution', with 100 normally distributed random numbers with mean = 4 and standard deviation = 0.5. We then considered three illustrative functions: 1) A simple shift, representing titre-independent boosting without waning: $f(x) = 1 + x$; 2) Titre-dependent boosting without waning: $f(x) = f(x) = 1 + e^{0.3x}$; 3) Titre-dependent boosting with waning: $f(x) = 2 + e^{0.3(x-2)}$. Note that these are illustrative functions selected to show the relevant dynamical properties, and so results will not be sensitive to specific parameters or titre distributions chosen as long as the $|f(x^*)|<1$ criteria are the same.

Missing data

While the overall dataset was near complete, there were a small number of missing data including for education level (N = 1), area of residence (N = 4), and occupation (N = 6). Missing data for regression models, partial analyses, and PCA were addressed using multiple imputation with the *mice* package in R. This involved creating five complete datasets (m = 5) from the original data using the *mice* function. This imputation process accounts for the uncertainty associated with missing values. The *mice::complete* function was used to extract a single, complete dataset from the set of five imputed datasets created by mice. This was then used for subsequent analyses.

Data sources

National and provincial demographic data and cluster population and classification (urban versus rural) were provided by the Dominican Republic National Statistics Office and the United National Statistics Division.[11,12] SARS-CoV-2 cases and deaths were obtained from COVID-19 GitHub repository.[16] Data on COVID-19 vaccinations are available from https://ourworldindata.org/coronavirus. Other data were enumerated during the study.

Role of the funder

The study was primarily funded by the Centers for Disease Control and Prevention (CDC) under grant U01GH002238, with salary support provided by the Wellcome Trust under grant 206250/Z/17/Z, and the Australian National Health and Medical Research Council under Investigator grant APP1158469. The funders, specifically the CDC, were involved in the design of the study, data collection, analysis, and interpretation of data. Additionally, CDC staff contributed to the manuscript's editing process. The Wellcome Trust and the Australian National Health and Medical Research Council provided salary support to AK and CL, respectively, but did not have direct involvement in the study's design, data collection, analysis, interpretation of data, or manuscript preparation.

Results

Study design and participants

Of the 6683 individuals enrolled in the multistage national serological survey, 1045 individuals from 38 clusters (in prespecified study regions in the northwest and southeast) were resampled between 28 Oct and 29 Nov 2022 (Fig. 1A–C). 1026 individuals had paired samples suitable for serological analysis and are included in the current study (Supplementary Fig. S1), with details of study participants described in Supplementary Table S1. COVID-19 vaccine coverage was high with 83.9% (95% Confidence Interval 81.5%–86.0%) having received at least one COVID-19 vaccine at

the first sampling timepoint, increasing to 93.0% (CI 91.3–94.4) by the second timepoint. Just 43.6% received a vaccine dose between the sampling timepoints and the mean number of doses increased from 1.7 to 2.3 doses per person. The median interval since the last vaccine dose received was 71 days (IQR 11, 131) at the first sampling timepoint and 437 days (IQR 334, 503) at the second sampling timepoint (Fig. 1D, Supplementary Table S2), reflecting the national slowdown in vaccine uptake (Fig. 1B).

Dynamics of population immune marker distribution

S-antibody prevalence between sampling time points increased from 92.8% (91.0–94.2) to 100%, affirming high baseline exposure and indicating all participants were exposed through infection, vaccination, or both by the second sampling timepoint. While only 44% of participants received a vaccine dose between sampling timepoints, 80.6% (CI 78.1–82.9) registered an increase in titres. S-antibody GMT values increased 9.9-fold (Supplementary Fig. S2 and Table S3, Fig. 1), suggesting widespread infections during the three waves of interval transmission, attributable to Delta, BA.1, and/or BA.2/4/5 (Fig. 1). To assess if infection or vaccination drove the increase in antibody levels, we measured changes among those that did not receive an interval COVID-19 vaccine dose and 77.5% (CI 74.0–80.8) registered an increase in antibody levels between sampling timepoints (considered a surrogate measure of interval infections, in the absence of interval vaccination). GMT titres increased 6.6-fold (Fig. 2A, Supplementary Fig. S1). We then assessed S-antibody changes among individuals that had never received a COVID-19 vaccine. 95.8% (CI 87.6–98.7) registered an interval increase and GMT titres increased 43-fold (Supplementary Fig. S1). Overall, these findings confirm a substantial cumulative force of infection over the study period.

Notably, we observed a marked normalization of the S-antibody titre distribution across the study population, from a broad and largely irregular distribution during mid-pandemic (Aug 2021, sampling midpoint) to a near Gaussian single-peak distribution by late-pandemic (Nov 2022) (Fig. 1F). This finding was consistent regardless of age or number of COVID-19 vaccine doses received (Fig. 1G), and by study region, suggesting the driver of this observation was likely generalizable. We considered that normalization of titre distribution to a single peak may be secondary to titre-dependent boosting, wherein boosting between the sampling timepoints occurs in a non-uniform manner dependent on baseline titres. To explore this, we examined individual changes in antibody levels, and observed a consistent increase in interval antibody levels among those with lower baseline titres and declining titres among those with higher baseline titres (Fig. 2A and B).

To better characterize the relationship between S-antibody levels at the two sampling timepoints, we used generalized additive models (GAMs) to account for factors that may be associated with differential exposure risk or rate of antibody decay and demonstrated a near linear inverse relationship between baseline S-antibody level and the change in antibody level between the timepoints (Fig. 2C, Supplementary Table S4), with similar findings when stratified by number of interval vaccine doses (Fig. 3). We then stratified by study region (NW versus SE) and found similar results (Supplementary Fig. S3) supporting the generalizability of these observations, at least across the Dominican Republic. Unadjusted analyses to assess the crude relationship between baseline S-antibody levels and subsequent antibody changes were similar (Supplementary Fig. S4).

We then explored the underlying relationship between key covariates known or suspected to influence the level of immune markers using principal component analysis to assess if differing computation approaches generated consistent findings. We again identified a strong inverse relationship between baseline S-antibody level and the change in antibody levels between the sampling timepoints, with opposing vectors between baseline S-antibody levels and interval S-antibody change indicating an inverse correlation (Fig. 2D). Notably, the number of interval vaccine doses, which we considered may drive interval S-antibody dynamics, exhibited a modest contribution to S-antibody change. PCA findings were similar between study regions (Supplementary Fig. S5), implying that the relationship between the change in S-antibody level and the other key covariates were similar irrespective of study site.

Prediction of change in antibody levels

To assess if our primary GAM models were robust, we utilized three out-of-sample techniques to assess their predictive performance. All three methods achieved comparable and high predictive performance (Pearson correlation between predicted and observed value, all 0.93) (Fig. 2E, Supplementary Table S6). Considering the considerable association between baseline S antibody levels and changes in antibody levels, we ran a variation of the model that included only baseline S antibody levels as a predictor variable which demonstrated similar high predictive performance (correlation coefficients all 0.92) (Supplementary Table S6).

Sensitivity analyses

Given the unexpected finding that mid-pandemic S-antibody levels were strongly associated with the individual-level change in antibody titres measured in the late-pandemic phase, irrespective of other factors including the number of vaccine doses received between the two sampling timepoints, we conducted sensitivity analyses to assess for alternate explanations. First, we

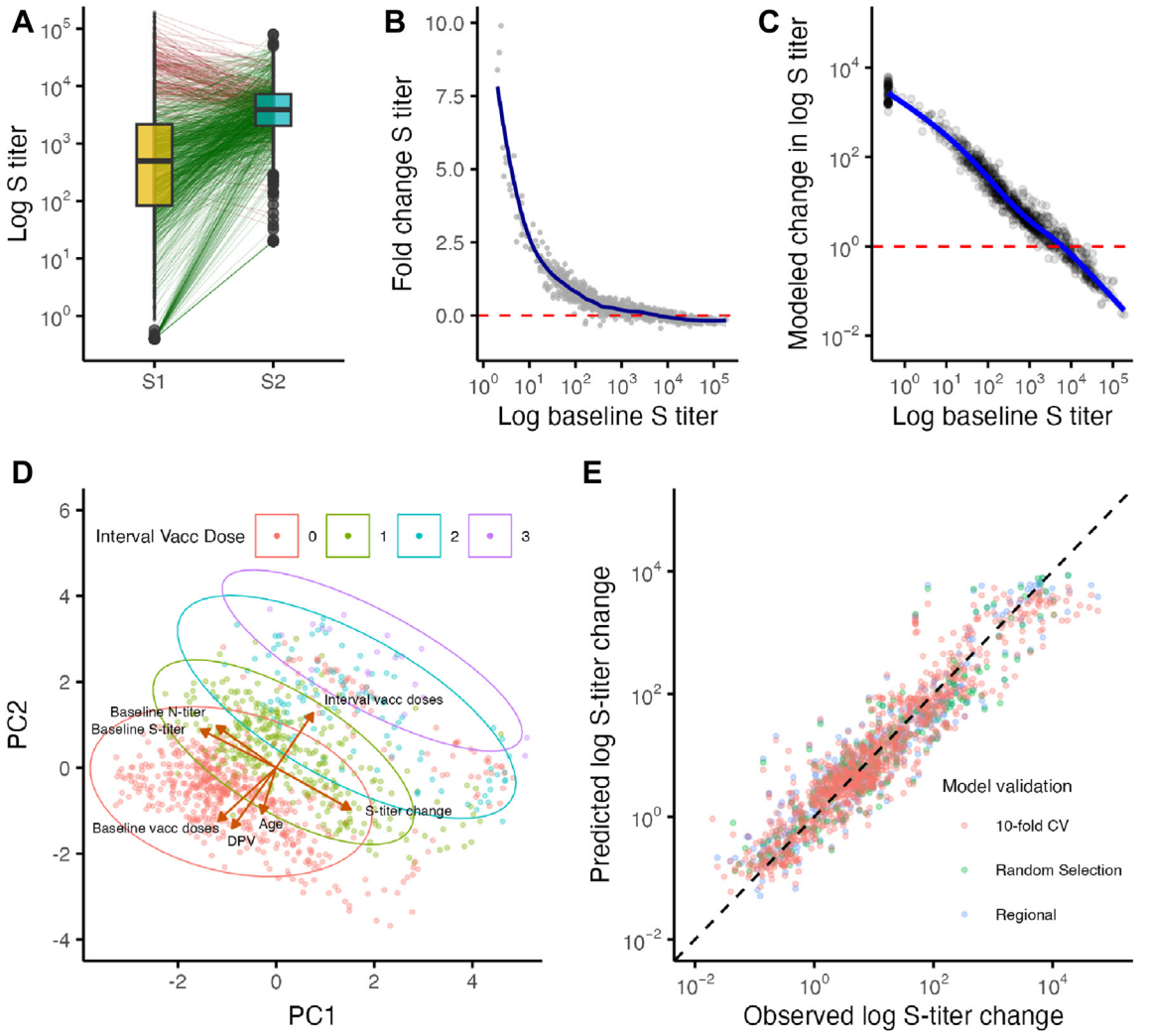

Fig. 2: **Observed and modeled changes in SARS-CoV-2 spike antibody changes following waves of Delta, BA.1, BA.2, and BA.4/5 transmission. (A)** Paired boxplot show S-antibody levels at the first (S1) and second (S2) sampling timepoints. The thick horizontal line indicates the median value, with lower and upper hinges representing the first and third quartiles. Whiskers indicate values between the first quartile and the lowest value, and third quartile and highest value, respectively, but no further than the 1.5 x the interquartile range. Values outside this range are plotted individually. The thin lines connecting time periods indicates changes in individual participant antibody levels with red lines representing a decline in S-antibody level and green lines indicating an increase in S-antibody level. All samples are paired across the sampling periods (n = 1026). **(B)** Scatterplot shows observed (unadjusted) fold change in log10 antibody level (y-axis) by baseline S-antibody level (x-axis) (n = 924). Log values < 100 not shown (N = 102). Blue line represents LOESS smoothing and dashed red line indicates no difference in baseline and follow-up S-antibody level. **(C)** GAM estimates of the change in antibody level between sampling periods (y-axis) by antibody level at the first sampling time point (x-axis). The regression curve is indicated with the blue line with shading indicating the 95% CI and individual regression partial values with gray dots. The horizontal red dashed line represents no antibody change between sampling timepoints. To control for variables that influence or may influence exposure risk or differential antibody contraction, the model included age, sex, urban versus rural setting, number of COVID-19 vaccine doses prior to the first sampling period (S1), days since last vaccine dose, and days between samplings. N = 1026. Model output and metrics detailed in Supplementary Table S4. **(D)** PCA biplot shows the relationships between the underlying data with colored ovals representing the 95% confidence regions (area approximating 95% of the data points) for the number of interval vaccine doses. The light green circle represents the 95% confidence region for all data points. Points represent observations, and arrows indicate the contribution of the variables to the principal components. Longer arrows suggest stronger correlations, and arrows pointing in the same direction indicate the corresponding variables have similar patterns of variation. Proportion of variance explained is 40.5% and 22.6% for PC1 and PC2 respectively, with full listing in Supplementary Table S7. N = 1024. **(E)** Scatterplot of predicted versus observed log10 S-titre change from the GAM model shown in plot C using three out-of-sample validation approaches (N = 1026) with model metrics listed in Supplementary Table S6.

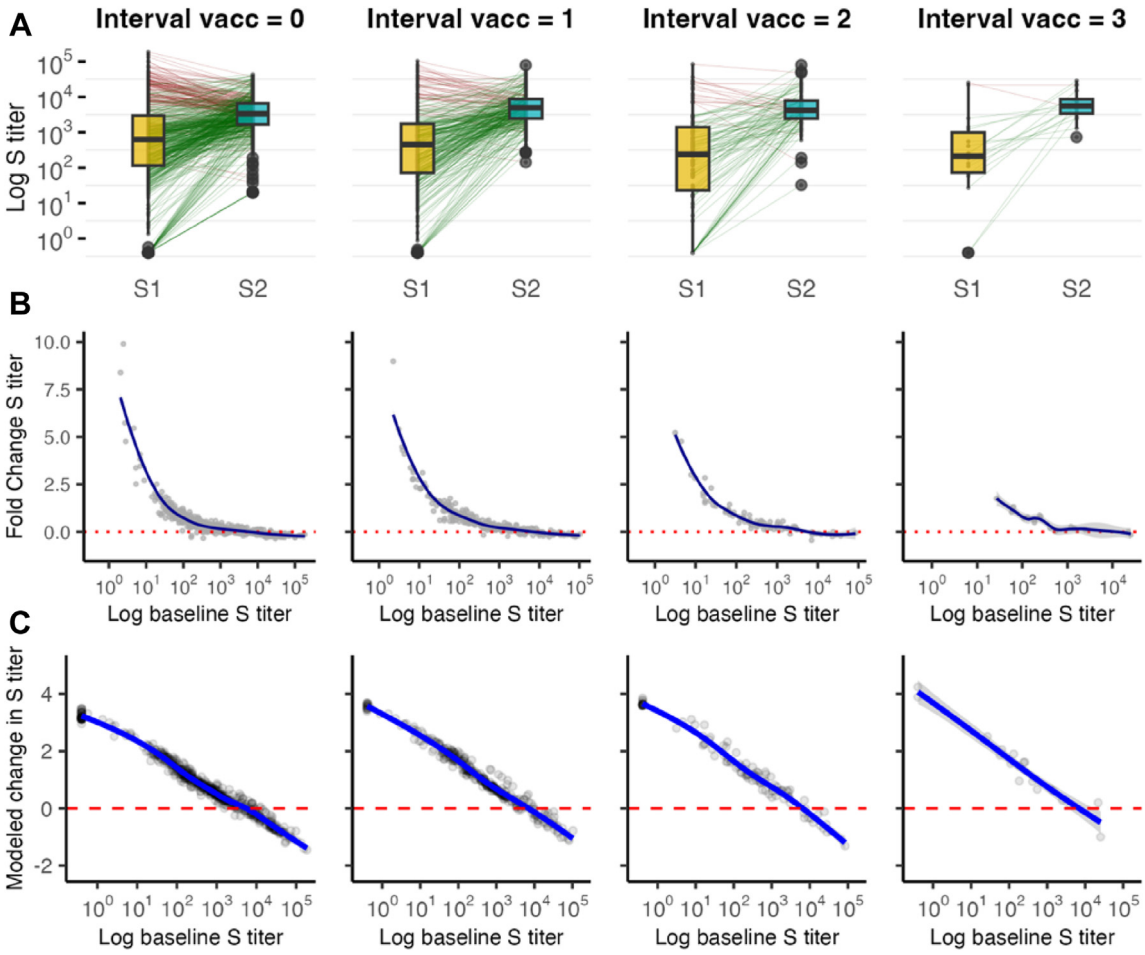

Fig. 3: **SARS-CoV-2 spike antibody changes by number of interval COVID-19 vaccine doses received.** Baseline and follow-up sampling midpoints were Aug 2021 (S1) and Nov 2022 (S2). Top row shows paired boxplots of S-antibody levels for each sampling period, stratified by number of interval COVID-19 vaccine doses (i.e., the number of doses received between the first and second sampling periods). The thick horizontal line indicates the median value, with lower and upper hinges representing the first and third quartiles. Whiskers indicate values between the first quartile and the lowest value, and third quartile and highest value, respectively, but no further than the 1.5 x the interquartile range. Values outside this range are plotted individually. The thin lines connecting time periods indicates changes in individual participant antibody levels with red lines representing a decline in S-antibody level and green lines indicating an increase in S-antibody level. All samples are paired across the sampling periods. **(B)** Scatterplot demonstrates observed (unadjusted) fold change in antibody level (y-axis) by baseline S-antibody level (x-axis), stratified by number of COVID-19 vaccine doses. Black line represents LOESS smoothing and dashed red line indicates no difference in baseline and follow-up S-antibody level. Log values < 100 not shown. **(C)** GAM estimates of the change in antibody level between sampling periods (y-axis) by antibody level at the first sampling time point (x-axis). The regression curve is indicated with the blue line with shading indicating the 95% CI and individual regression partial values with gray dots. The horizontal dashed red line represents no antibody change between sampling timepoints. To control for variables that influence or may influence exposure risk or differential antibody contraction, the model included age, sex, urban versus rural setting, number of COVID-19 vaccine doses prior to the first sampling period, days since last vaccine dose, and days between samplings. Summaries of model outputs are provided in Supplementary Table S4. Individuals that received four interval vaccine doses (N = 2) are not shown. Number of study participants for the top and bottom rows are 579 (interval vaccine = 0), 322 (interval vaccine = 1), 105 (interval vaccine = 2), 18 (interval vaccine = 3). Data for four interval vaccine doses (n = 2) are not presented. Number of participants for the middle row, by plot are 528 (interval vaccine = 0), 292 (interval vaccine = 1), 84 (interval vaccine = 2), and 18 (interval vaccine = 3). The difference in observation number between the middle versus the top/bottom rows is because participants with log values < 100 are not shown for plots in the middle row.

considered the possibility of regression to the mean—a well-documented phenomenon where initial extreme measurements tend to converge to the mean in subsequent measurements. Using a permutation approach[17] we found no significant association between shuffled initial S-antibody levels and subsequent ones (p-value 0.935, R-squared was nearly zero), suggesting that regression to the mean did not meaningfully influence

our results. Then, we excluded outliers and carried out-of-sample analyses and compared results with all data versus data excluding outliers. The findings were essentially unchanged, with a difference in R-squared value of 0.004. Finally, we evaluated the relationship between baseline antibody levels and their subsequent changes across the entire spectrum of baseline values. This was to assess for evidence of a ceiling effect arising from potential instability in assay measurements for very high baseline values. Our analysis showed no significant deviation for high baseline antibody levels from the general trend, indicating that a ceiling effect was unlikely to influence our results (Fig. 2B).

Drivers of S-antibody titre change between sampling timepoints

After demonstrating that S-antibody levels at the baseline sampling timepoint tracked closely with the interval change in antibody levels, we sought to better understand the role and importance of other key variables. To do this, we first performed partial dependence analyses using a random forest model and included baseline S-antibody and N-antibody titres, age, vaccine doses received prior to the first sampling timepoint, vaccine doses received between the first and second timepoints, and the number of days since the last vaccination dose (DPV) (Supplementary Fig. S6). The most important feature was baseline S-antibody titre, as indicated by its largest percentage increase in mean squared error (Inc. MSE, 136.4%) when permuted (Supplementary Table S5). Baseline N-antibody titre, days post vaccination, and interval vaccination doses were of low importance (Inc. MSE, 28.6%, 20.7 and 19.7 respectively), and baseline vaccination doses and age were minimally important (Inc. MSE, 8.1 and 7.5). These findings demonstrate that while multiple variables contribute to the trajectory of individual S-antibody levels, baseline S-antibody level (and therefore baseline immune protection) is the primary driver. Patterns of variation identified in the PCA analysis aligned with partial dependence analyses (Fig. 2D, Supplementary Fig. S6), indicating that irrespective of computation approach findings were similar.

Evolution of humoral markers to an immune setpoint

Two findings were unexpected and hard to reconcile with what is known about the complexity of population immunity and the immune response to vaccination. First that baseline antibody levels were strongly (but inversely) correlated with subsequent levels, and second that the number of interval vaccine doses did not appear to markedly impact later antibody levels. To reconcile these findings, we hypothesized that immuno-ecological pressure drives immune markers levels to a discrete setpoint below which antibody levels are boosted and above which antibody levels decline. To examine this hypothesis, we examined the change in antibody levels in more detail and indeed demonstrated a distinct level or "immune setpoint" around which antibody titres converge regardless of baseline levels and only marginally impacted by the of the number of vaccine doses received between the sampling timepoints (Fig. 4), with findings consistent when stratified by age and study region (Supplementary Fig. S7). These findings align with our prior analyses (Fig. 2A–D) that suggested a consistent convergence of antibody levels to a set point irrespective of baseline antibody levels.

Convergence of titres as a dynamical system

To further explore the immune setpoint concept, we considered plausible theoretical reasons for this pattern. As described in the methods, given a predictable relationship between pre-infection titre and post-infection titre, it is possible to define kinetics over multiple waves as a mathematical dynamical system. We found that if the change in titre post-infection depends on pre-infection titre and wanes for higher titres, then boosting and waning in antibody kinetics over multiple infection waves will gradually converge to a narrow distribution of set point titres (Fig. 5). This provides a theoretical explanation for the patterns observed in Figs. 2–4; if the relationship between pre- and post-infection titres is a convergent function, we would expect convergence to a set point, and initial titre and prior exposure history become less important in predicting titre over sequential exposures.

Discussion

Our study findings, derived from a carefully sampled population enrolled through a multistage household survey in the Dominican Republic, provides insights into the evolution of population immunity. We observed a convergence in the distribution of population immune markers to a single peak over time, provide evidence that this is driven by a process of titre dependent boosting, and demonstrate that antibody markers coalesce around a discrete setpoint. Collectively, these findings suggests that the accumulation of population immunity is not due to a random or semi-random series of vaccination and infection events. Rather, that the evolution of population immunity follows a predictable and non-random pattern, at least for pathogens such as SARS-CoV-2 that generate intense and widespread transmission.

While many studies have measured immune markers across populations, the examination of how immunity evolves across populations is largely unexplored. Our findings suggest that the introduction of widely transmitted pathogen into a susceptible population leads to a predictable evolution of immune markers. Other data supports this assumption. We previously reported on an independent study that

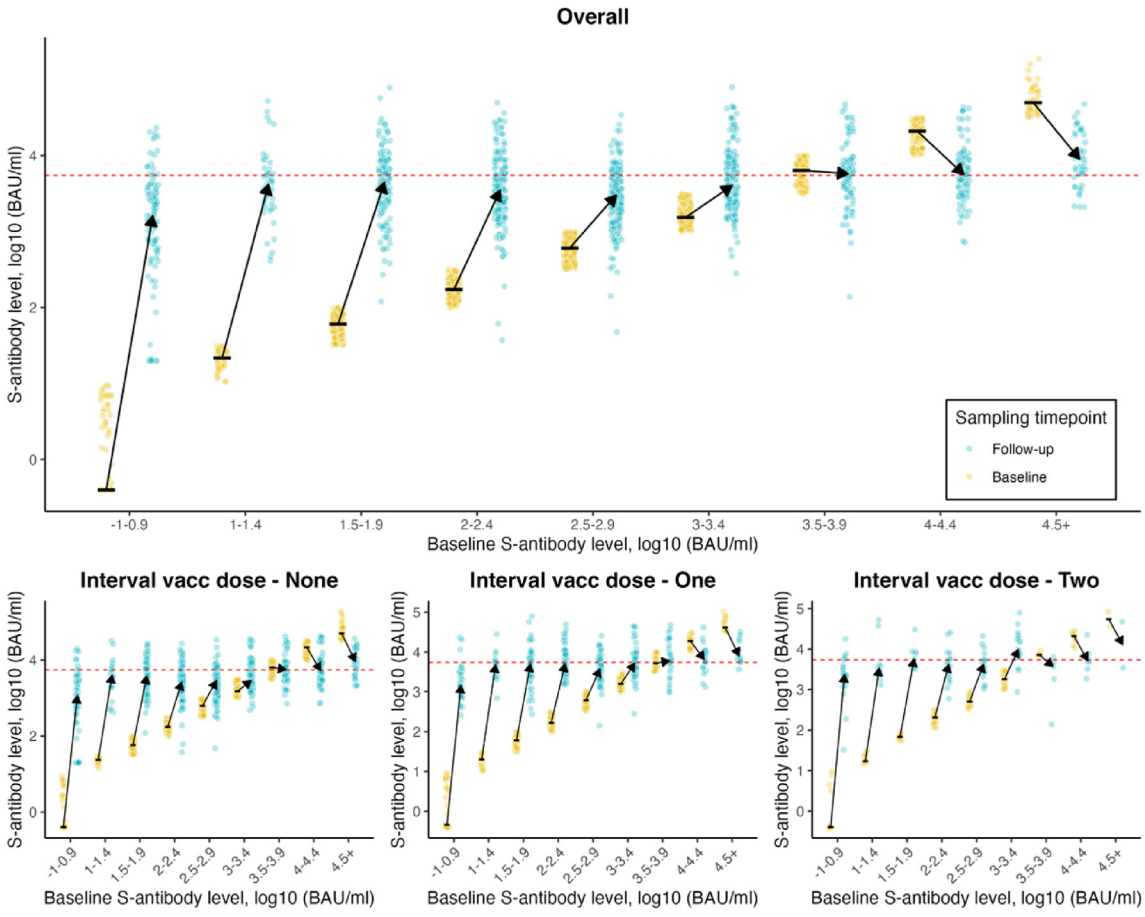

***Fig. 4:* Change in SARS-CoV-2 spike antibody levels by baseline titres.** Scatterplots of paired antibody levels are stratified in each plot by baseline antibody level, shown as a categorical variable on the x-axis. Horizontal black bar represents the median values at the first (baseline) sampling timepoint and arrowhead tip the median value at the second (follow-up) sampling timepoint. Connecting black line represents the change in median values between the sampling timepoints. Horizontal red dashed line is used illustratively as population "set-point" (3.75 BAU/ml) following the last SARS-CoV-2 infection wave (BA.2/4/5, Fig. 1). Main plot represents all data and lower plots represent data stratified by the number of COVID-19 vaccine doses received between the two sampling timepoints. Number of study participants by plot are 1026 (Overall), 579 (interval vacc dose = none), 322 (interval vacc dose = one), 105 (interval vacc dose = two). Data for three and four interval vaccine doses not shown given sparsity of data points (N = 18 and N = 2 respectively). Interval vacc dose indicates the number of COVID-19 vaccine doses received between the first and second sampling timepoint.

examined S-antibody levels among patients presenting to health facilities in the Dominican Republic with undifferentiated febrile infection, and demonstrated a similar evolution of SARS-CoV-2 titres from an irregular distribution to a single peak from mid to late pandemic (Supplementary Fig. S8).[5] In that study, the shift from an irregular to a regular distribution of antibody markers was progressive over time and was not due to, for example, a single wave of intense transmission. In South Africa, which has been heavily impacted by sequential waves of transmission,[10] a prospective cohort study documented similar trends with immune marker boosting among those with low baseline levels and waning among those with high baseline levels across Beta, Delta and Omicron waves of transmission.[18]

Predicting changes in individual-level immunity remains complex due to range of factors that influence the immune response. However, our findings suggest that when aggregated across a population, immune markers appear to follow distinct patterns. We developed a model trained on one group of study participants and, unexpectedly, were able to accurately predict changes in baseline S-antibody levels (a marker of immune protection) in an out-of-sample group more than a year later. The accuracy of the prediction was based almost entirely on the initial S-antibody level, overshadowing the anticipated influence of factors like additional vaccine doses, diverse exposure risks, waning immunity, and individual immune variations. In particular, while additional COVID-19 vaccine doses were associated with

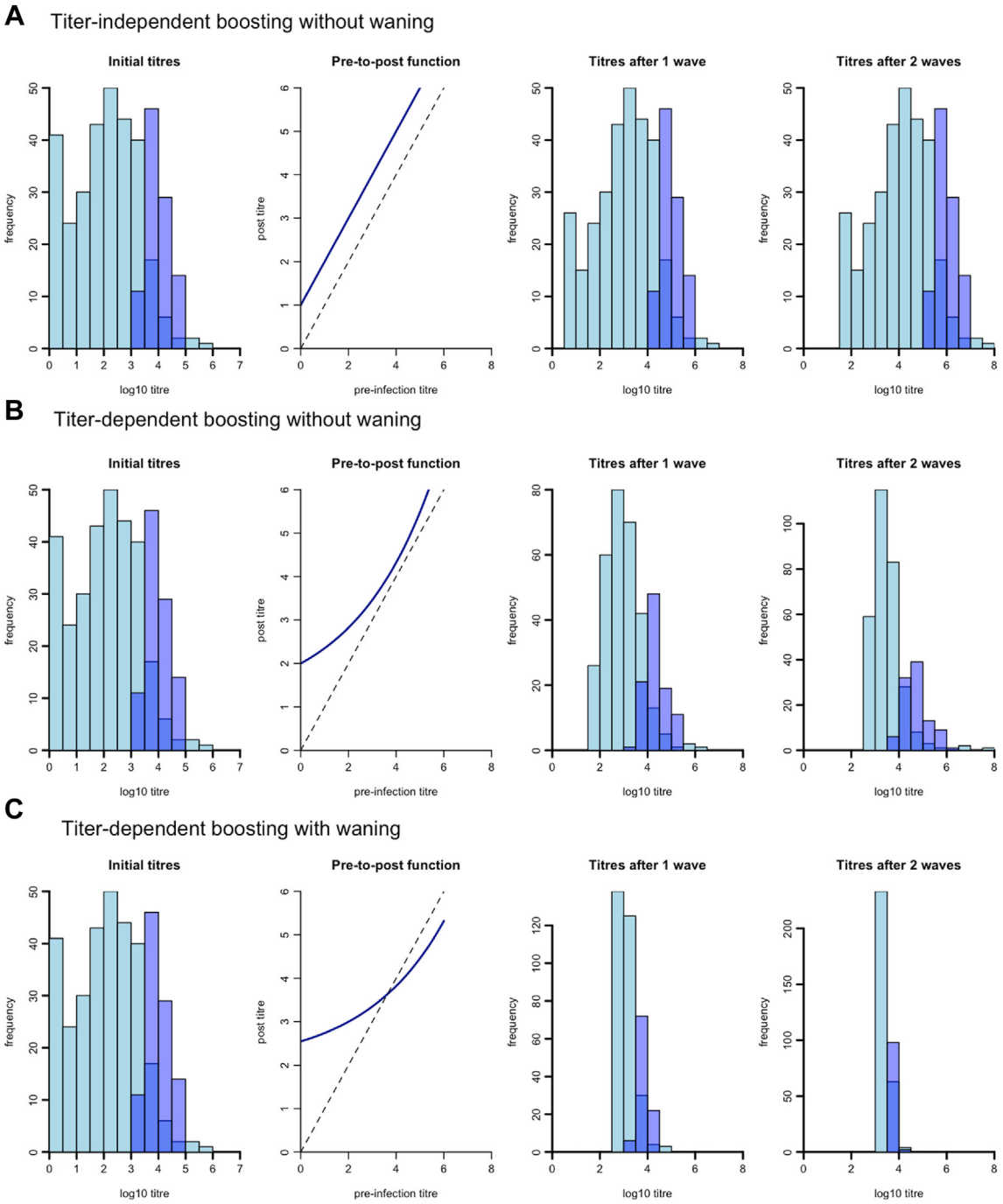

Fig. 5: **Theoretical insights into titre stability through simulated infection cycles.** Plots demonstrate the relationship between initial titres and titres after 1 and 2 waves of infections for different functional relationships between pre-infection and post-infection titres. The first column represents baseline titres for two hypothetical titre distributions (light blue and purple). Different distributions were intentionally chosen to illustrate how the specified functions impacted markedly different distributions. Column two illustrates the function applied to the baseline titres. **(A)** Uniform boost post-infection, independent of prior titre, and without waning. This results in a non-convergent dynamical system with no fixed point, and hence titres would be expected to increase over time. **(B)** Titre-dependent boost with no waning. This results in a non-convergent dynamical system, with gradually increase and narrowing titre distribution. **(C)** Titre-dependent boosting and waning for higher titres, which produces a dynamical system with a stable fixed point, and hence convergence in titres to a set point over multiple waves, regardless of initial value.

a larger increase in S-antibody levels between the sampling timepoints, the impact was less pronounced than anticipated. Despite the fact that vaccinations undeniably bolster the humoral response, their role in directing overall population immune dynamics seems less influential than previously assumed in this context.

While the limited influence of vaccinations on subsequent immune marker levels is perhaps counterintuitive, it provides valuable insight into the likely drivers of population-level immunity dynamics. Our data strongly suggests the existence of a population immune setpoint, a level of antibody response towards which individuals and ultimately the population converge (Fig. 2). The pressure from population-level infection waves and titre-dependent antibody kinetics to reach this setpoint appears to override individual factors like additional vaccine doses, implying that irrespective of whether antibody responses stem from infection or vaccination, similar levels of response—and hence protection—are ultimately attained in high transmission settings. Notably, this does not undermine the role of vaccines, rather the opposite. Although vaccines did not drive markedly higher titres when measured over the entire study period, we interpret that this reflects their role in reducing infection. If infection-induced boosting was uniform across the population (i.e., similar rates of infection among those that did versus did not receive interval vaccine doses), those that received additional vaccine-induced boosting would, under most circumstances, be expected to register the largest increases in antibody titres. The fact that this was not the case, and similar levels of boosting were observed regardless of vaccine doses received (Figs. 2 and 4), implies that infections, and therefore infection-induced boosting, were less common among vaccinated versus unvaccinated individuals.

Similar to the finding that additional vaccine doses did not substantially impact later antibody levels, our finding that baseline antibody levels were strongly associated with changes 15-months later was not initially anticipated. Given the multiple factors that would be expected to disrupt future antibody level prediction we considered it highly unlikely that a baseline antibody measurement would independently (i.e., without carefully adjusting for individual-level factors that influence boosting and waning) predict the change at follow-up measurements more than a year later. However, when considered in the context an environment that drives antibody levels to an immune setpoint, this is precisely what would be anticipated. Given coalescence to a set point, individuals with low baseline antibody levels would be expected to register the largest increases while those with high levels would be expected to register the smallest increases or declining values, as we observed.

While immunological setpoints have been described for individual responses to malignancies or immunotherapies, and for population-level humoral responses following vaccinations, the concept of a population immunological setpoint driven by ecological pressures has not been previously reported. We encourage further independent investigation of this phenomenon, which has a range of potential public health implications. For example, we identified an immunological setpoint of around $10^{3.7}$ BAU/ml (or 5500 BAU/ml) (Fig. 2), a titre that translates to an estimated 90–95% reduction in relative risk of symptomatic SARS-CoV-2 infection from BA.2 and BA.4/5, based on correlates of protection estimates using the same immunoassay.[19] This raises the question of whether population immune setpoints consistently track with measured immune correlates of protection, a seemingly logical hypothesis assuming sufficiently widespread transmission. If so, immune correlates of protection that can be estimated relatively quickly,[19] could serve as a valuable tool for estimating future population immune setpoints, with public health implications such as tailored vaccine strategies for high risk populations and refined model estimates of future transmission. Furthermore, our finding that the distribution of immune markers shifts to a single normalized peak over time may allow for more efficient serological surveys by requiring fewer data points compared to sampling an irregular or skewed distribution, given the same power calculation. Yet, further work is necessary to determine how and when streamlined sampling would be appropriate. Additionally, the shift of immune markers to a normal distribution may prove be a useful biomarker signal of transition from epidemic to endemic transmission, acknowledging that defining endemicity is challenging for rapidly evolving pathogens. While the current study focuses on SARS-CoV-2, the normalization of immune markers and the immune setpoint phenomenon may inform the epidemiological investigation and public health response for other widely transmitted pathogens.

Strengths of this study include a sampling approach that was carefully designed and implemented using a three-stage methodology to maximize representativeness. All participants provided paired samples for serological analyses which allowed us to track individual as well as population level antibody changes over time. We analyzed data from two geographically discrete study locations in the northwest versus southeast of the country to understand if regional differences impacted our findings. We employed a widely used and extensively validated immunoassay that has been demonstrated to track with immunological protection. Finally, we used a range of computational and sensitivity analysis approaches to triangulate and validate our findings. Yet there are some limitations. We measured antibodies against the spike protein of the ancestral virus, rather than the spike of more recent variants. However, several studies have demonstrated that boosting after exposure to variant antigens primarily generates cross-reactive antibodies with minimal if any variant-specific

antibodies.[8,20,21] Given the current study population were highly exposed through infection, vaccination, or both, prior to the first sampling timepoint (i.e., prior to Delta and Omicron) subsequent infections can be assumed to largely backboost based on prior immune imprinting. While we clearly demonstrate that titre-dependent boosting drove the evolution of immune markers over multiple waves in this settings, our data which did not include testing for acute infection cannot definitively establish the reason for this pattern of boosting. Given that multiple immune correlates of protection studies have demonstrated that infection risk tracks closely with immune titres we assume that immune restricted transmission is the primary driver, wherein those with high titres avoid infection and those with lower titres are infected.[19,22–24] However, other factors may contribute. For example, some data suggests that the magnitude of boosting and rate of waning generally decrease following multiple exposures.[8] Similarly, prior studies on influenza have demonstrated that boosting after vaccination is attenuated among individuals with high pre-vaccination haemagglutination inhibition assay titres, when compared against those with low pre-vaccination titres.[25] These findings could potentially contribute to a similar pattern of immune markers evolution as we observed. While we used a total S-antibody assay for our study, the underlying principle suggests that similar dynamics would be observed for any humoral marker that tracks with protection against infection. Although sampling was conducted using a multistage approach with findings similar across discrete geographic regions, our conclusions may not apply to other settings. Lastly, while our data does not explicitly examine the role of emerging viral variants, the principle of a pathogen or variant-specific setpoint would be expected to apply regardless of viral characteristics given sufficient transmission and an immunoassay that tracks with infection risk.

In conclusion, our study provides unusual insights into the immunoepidemiology of SARS-CoV-2 in a high transmission setting. We observed titre-dependent differences in individual antibody kinetics that led to the convergence of immune markers to a population immune setpoint. The selective force driving population immunity towards this setpoint appears to be sufficiently strong to minimize the impact of a range of factors typically considered important for eliciting or modulating the humoral response. While further investigation is needed to fully understand the drivers and implications of these findings, the influence of varying transmission intensity and emerging strains, and the consistency of immune correlates in predicting setpoints, our study offers a preliminary blueprint for how population immunity evolves under high transmission pressure. This knowledge may be leveraged to decipher transmission patterns, predict epidemic risks, and guide public health policy for SARS-CoV-2 and potentially other pathogens.

Contributors

EJN led the conceptualization, study design, funding acquisition, data interpretation, visualization, writing of the original draft, and overall supervision of the project. KR was responsible for data collection, methodology, writing review & editing, and project administration. MdSA and SG contributed to data curation and writing review & editing. HM, ACR, and BL supported the methodology and writing review & editing. DD, MCE, WD, FP, and GA were involved in data collection and writing review & editing. PJ, TO, LdlC, IMS, and KM carried out the investigation and participated in writing review & editing. MB and RSR managed project administration and manuscript review and editing. CTP participated in data collection, project administration, and writing review & editing. CLL contributed to methodology, funding acquisition, and writing review & editing. AK contributed to methodology, funding acquisition, simulation model development, data interpretation, and writing review & editing. All authors read and approved the final version of the manuscript. EJN and AK accessed and verified the underlying data.

Data sharing statement

A complete de-identified dataset and R code are available at https://github.com/enilles1/immunoepidemiology for the purpose of reproducing and building on the analyses.

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the US CDC.

Declaration of interests

EJN is the PI on a US Centers for Disease Control and Prevention (CDC) funded U01 award that funded the study. AK and CLL are co-investigators on the same award. DD, MdSA, SG, MCE, WD, GA, MB, and KWR have received salary support, consultancy fees, or travel paid through this award. BL is an employee of the US CDC. CTP, LC, IMS and RSR are employees of the Ministry of Ministry of Health and Social Assistance, Dominican Republic, that was subcontracted with funds from the US CDC award. AK is supported by the Wellcome Trust, UK. CLL is supported by the Australian National Health and Medical Research Council. CDC staff supported the design and manuscript editing. We declare no other competing interests.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2024.105319.

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