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Vaccination and hybrid immunity is associated with SARS-CoV-2 protection but not shedding duration in rural Guatemalan households

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LIST OF ABBREVIATIONS

AGRI-CASA, Agricultural Worker COVID-19 Asymptomatic and Symptomatic Transmission in the Home and Workplace Study; CLI, COVID-19-like illness; COI, cut-off index; NAb, neutralizing antibody; CU-AMC, University of Colorado Anschutz Medical Campus; IC₅₀, 50% inhibitory concentration; IQR, interquartile range; POC, point-of-care; RT-PCR, reverse transcriptase polymerase chain reaction.

ABSTRACT

Background: Essential agricultural workers in Central America and their households faced a high force of infection during the COVID-19 pandemic. Here, we evaluated if pre-existing seropositivity and vaccination (hybrid immunity) influenced SARS-CoV-2 infection risk and duration of shedding in an agricultural community in rural Guatemala.

Methods: Individuals from 70 households were enrolled in a community cohort. From September 2021 to December 2022, all participants reported symptoms twice-weekly and provided saliva weekly for SARS-CoV-2 RNA testing by reverse transcriptase polymerase chain reaction (RT-PCR). Upon SARS-CoV-2 detection, participants submitted saliva three times per week for four weeks, which was tested by RT-PCR, to assess duration of shedding. We selected 119 SARS-CoV-2 PCR-positive individuals and 101 month-matched controls to measure nucleocapsid IgG and Omicron neutralizing antibody (NAb) titers.

Results: Compared to uninfected controls, SARS-CoV-2 cases were more frequently female (60.5% vs 48.5%, $p=0.08$), had fewer vaccine doses ($p=0.07$), and were less likely to have hybrid immunity ($p=0.08$). Compared to controls, SARS-CoV-2 cases had lower median titers of nucleocapsid IgG (cutoff index [COI]=9.6 vs 23.6, $p=0.12$) and Omicron NAb (28.4 vs 50.4, $p=0.15$). SARS-CoV-2 cases also had lower NAb than controls among those vaccinated with 1 (16.1 vs 182.7, $p=0.01$), 2 (11.9 vs 87.0, $p=0.04$), or 3 doses (13.4 vs 232.5, $p=0.004$), and those considered up-to-date on vaccination (13.1 vs 155.6, $p=0.0001$). Nucleocapsid IgG and Omicron NAb were similar between individuals with short (≤ 7 days) vs long (> 7 days) viral shedding durations.

Conclusions: In a household cohort of Guatemalan agricultural workers, cumulative vaccine doses associated with lower odds of infection. Among those previously vaccinated, lower titers

67 of nucleocapsid IgG and Omicron NAb were associated with elevated infection risk, highlighting
68 the importance of hybrid immunity in COVID-19 protection in this population.

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BACKGROUND

Essential workers in the agricultural sector and their household contacts may be at increased risk of SARS-CoV-2 exposure and transmission, due to occupational exposures and crowded living conditions (1, 2). Defining correlates of protective immunity against SARS-CoV-2 infection and COVID-19 disease in agricultural worker populations and their communities is important given their high risk of SARS-CoV-2 exposure and their importance in global food security. Though many studies have evaluated serologic immunity as a correlate of protection in diverse populations (3-12), primarily against COVID-19 disease, few studies have focused on agricultural communities, especially in low- and middle-income country (LMIC) settings with differing risks of exposure and disease severity (2, 13-15). Correlates of protective immunity in this highly exposed, essential worker population may be different than other risk groups and could be used to predict population-level susceptibility to future infection or as vaccine immunobridging studies (9, 16).

We previously characterized significant clinical and economic consequences of COVID-19 in a cohort of essential agricultural workers in rural southwest Guatemala (17, 18). Subsequently, we demonstrated a high force of infection in the households of a subset of those workers enrolled in the related “AGRIcultural worker COVID-19 Asymptomatic and SymptomAtic transmission in the Home and Workplace (AGRI-CASA) Study” (19). The AGRI-CASA study included extensive monitoring of household contacts of SARS-CoV-2 index cases that could provide insights on factors influencing human-to-human SARS-CoV-2 transmission. Here, we aimed to evaluate the protective effect of pre-existing serologic immunity and vaccination on subsequent

92 SARS-CoV-2 infection and shedding among household participants enrolled in the AGRI-CASA
93 study, encompassing successive infection waves due to Omicron and its subvariants.
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METHODS

The present study was a nested case-control analysis performed among a subset of participants who had enrolled in the parent AGRI-CASA cohort study (19). The AGRI-CASA study was conducted in two rural, resource-limited communities in the coastal lowlands of southwest Guatemala. (17, 20, 21); rolling enrollment included 70 households from the two communities. Households that ended participation were replaced to maintain the 70-household cohort. AGRI-CASA enrollment criteria included having ≥ 1 household member employed at a large agribusiness who participated in the separate AGRI study, residing within the two study communities, and at least 75% of household members consenting to participate.

Data Collection and Routine Surveillance

Following informed consent and assent, as appropriate, AGRI-CASA study nurses collected standardized demographic, clinical, and epidemiologic data, including household-level (number of adults/children, environmental data) and individual-level (sex, vaccination status, vaccination date, and self-reported medical conditions) characteristics. All enrolled participants were screened twice weekly for COVID-19-like Illness (CLI) defined as ≥ 1 of the following symptoms for ≥ 48 hours: fever/chills, cough, shortness of breath, fatigue, muscle/body ache, headache, loss of taste/smell, sore throat, congestion, nausea, vomiting, or diarrhea (22, 23).

Specimen collection and testing

Each week, regardless of symptom screening results, 5 ml of saliva was collected for future virologic testing. In addition to weekly saliva collection, participants with symptoms meeting CLI criteria or with confirmed community or household exposure to SARS-CoV-2 in the past three days received an additional visit for saliva collection.

Saliva samples from individuals with CLI or exposure were tested for SARS-CoV-2 by PCR within 24 hours. Subjects with negative PCR results returned to routine surveillance. For households with a SARS-CoV-2-positive swab, all enrolled household contacts provided detailed clinical and epidemiological data through six weeks. Household contacts of PCR-positive individuals also provided blood specimens, and saliva three times per week for four weeks, regardless of their symptom or laboratory results (19). Following the 6-week visit, households returned to routine surveillance.

SARS-CoV-2 PCR Testing

All saliva specimens were transported on ice, processed, and stored at -80°C within 24 hours, as previously described (19). Specimens were processed according to the saliva-direct protocol (24), mixing 200 µl of saliva with 400 µl of 0.9% saline then added to the Roche Cobas® assay tube. Saliva specimens from symptomatic individuals identified during routine surveillance were tested off-label within 24 hours (25) using the Roche point-of-care (POC) Cobas® Liat real-time PCR (RT-PCR) Influenza A/B & RSV and SARS-CoV-2 kits (26). POC PCR results were shared with participants within 24 hours. Specimens from households collected after the incident detection of SARS-CoV-2, influenza, or RSV were tested at a later date by RT-PCR testing (CDC Protocol (27)) at Universidad del Valle de Guatemala (UVG) or digital droplet PCR at Colorado State University (Fort Collins, CO) (28). Retrospectively, saliva collected from all household contacts during routine surveillance in the two weeks preceding the incident household viral detection were also tested. POC and batched RT-PCR results were shared weekly with the Guatemala Ministry of Health.

Selection of Cases and Controls for Sub-Study

“Cases” were defined as individuals of any age enrolled in the AGRI-CASA study with PCR-confirmed SARS-CoV-2 infection and a serum sample available at the University of Colorado-Anschutz Medical Campus (CU-AMC) collected during the 12 months preceding infection. “Controls” were individuals without SARS-CoV-2-confirmed infection who had a prior serum sample available at CU-AMC. Control serum samples were selected to match case serum samples by month of collection; for case:control pairs that did not have month-matched serum samples, control serum samples were selected so the median overall collection dates were similar between cases and controls (**Figure 1A**).

SARS-CoV-2 Serologic Testing

At the Santiago Laboratory at CU-AMC, serum was tested for anti-nucleocapsid IgG, reflective of prior infection, using the Roche Diagnostics Elecsys® anti-SARS-CoV-2 immunoassay, as previously described (18). All specimens were run in triplicate on the Roche Elecsys® e801 per the manufacturer’s instructions and reported as cut-off index (COI) value. The assay was previously reported to have a sensitivity of 95% and specificity of 99.8% at >14 days post-PCR positive SARS-CoV-2 patient samples (29).

To measure SARS-CoV-2 neutralizing antibodies (NAbs), we utilized a lentivirus-based assay as previously described (18, 30) (**Supplemental Figure 1A**). Briefly, HIV-1 Δ env with a nanoluciferase reporter (Dr. Paul Bieniasz, Rockefeller University) was pseudotyped with codon-optimized SARS-CoV-2 Spike based on the Omicron strain (B.1.1.529 or BA.1; Invivogen catalog number p1-spike-v11) in 293T cells (31). Pseudovirions ($\sim 3\text{--}4 \times 10^5$ relative light units,

RLU) were co-incubated with serial 5-fold dilutions of serum (from 1:10 to 1:31250) in 100 μ l complete media (F-12 Ham's Medium with 10% FBS) containing 10,000 A549-ACE2 cells for 1 h at 37°C (32). The virus-serum-cell mixture was then plated into 96-well white plates, lysed after 48 h with Nano-Glo substrate (Promega) and RLUs were measured in a Victor X5 luminometer (Perkin Elmer). 50% inhibitory concentrations (IC50) were calculated using a two-phase decay equation (GraphPad Prism; **Supplemental Figure 1B**).

COVID-19 Vaccination

Guatemala first received COVID-19 vaccine through the COVAX mechanism on March 11, 2021, with Pfizer and Moderna vaccines comprising the majority (19). COVID-19 vaccination dates, doses, and manufacturer data were collected directly from the Guatemalan Ministry of Public Health centralized database. Individuals with no evidence of vaccination were considered unvaccinated, and number of doses (up to 4 doses) was documented. We also categorized vaccination status based on whether the most recent vaccine dose (at or after completion of the primary series) was received during the past 12 months. "Hybrid immunity" was determined by inferring SARS-CoV-2 infection from anti-N IgG positivity (see below) and ≥ 1 vaccine dose. We carried out a separate analysis excluding individuals who had a vaccine dose between the serum sample collection and the symptomatic visit.

Index and Secondary Case Definition

An index or co-index case was defined as the first person(s) who tested positive for SARS-CoV-2 within a household. A secondary case was defined as a household contact who tested positive during 1–14 days after the index case date. For analysis purposes, a tertiary case was defined as

any household contact who tested positive 14–42 days after the index case. Forty-two days after the incident index case infection, the household was considered clear of infection and susceptible to subsequent infection. Number of days positive for SARS-CoV-2 was calculated as days between the first and last day of PCR-positive test results within the 42-day window.

Statistical analysis

Three major questions were assessed: (1) whether SARS-CoV-2 infection status was associated with prior vaccination; (2) whether SARS-CoV-2 infection status was associated with prior serum antibody positivity/titers in the context of vaccination; and (3) whether SARS-CoV-2 viral shedding duration was associated with preceding serum antibody positivity/titer and vaccination. For continuous variables, medians and interquartile ranges (IQRs) were calculated for individual-level characteristics stratified by prior infection status based on enrollment serum testing; means were calculated and compared via t-test, and categorical demographic variables were compared using chi-square test or Fisher's exact test, as appropriate. Analysis of cut-off index (COI) values for SARS-CoV-2 anti-nucleocapsid (anti-N) IgG and IC_{50} for SARS-CoV-2 neutralizing antibody titer was completed using Prism software. Analysis was completed using two-way ANOVA with post-test analysis, t-test, and correlation analysis. A COI value > 1 was considered positive for anti-N IgG, as specified by the manufacturer. Comparisons of anti-N IgG positivity rates between 2 groups were evaluated using a 2-tailed Chi-Square test in Prism (version 10.2.3). Simple linear regression of log-transformed values was used to determine if there was a correlation between Omicron NAb and anti-N IgG titers, as well as the relationship between these antibody markers and the number of vaccinations. To determine differences in IgG and NAb titers by demographic variables, median titers were calculated by group or subgroup, and

compared using the Wilcoxon two-sample test. Statistical analyses were conducted using SAS v.9.2 (Cary, NC).

Data availability

The data underlying this article will be made available on request to the corresponding authors.

Ethical Oversight

The study was carried out in accordance with the Declaration of Helsinki. The study was approved by the Colorado Multiple Institutional Review Board (COMIRB protocol #21-2551), the UVG Ethics Committee (#225-01-2021), and CDC (#212551) as human subjects research. The local SW Trifinio Community Advisory Board for Research agreed to the study. All study participants provided written informed consent.

RESULTS

Between September 28, 2021, and December 21, 2022, 382 participants were enrolled in the AGRI-CASA cohort. Of these individuals, 263 participants had serum samples available at CU-AMC. Of these individuals, we identified 119 SARS-CoV-2 cases (**Figure 1A**), with the majority (n=95, 79.8%) of these serum samples collected during the start of the Omicron (BA.1) wave in Guatemala (November 2021) and extending towards the XBB subvariant wave, based on deposited sequences in GISAID.org and genomic surveillance of within the cohort (**Figure 1B**). An additional 29 SARS-CoV-2-positive individuals did not have a serum sample available prior to infection, and 14 had a sample available >12 months preceding their infection. Of the 119 SARS-CoV-2 cases, 38 (31.9%) met the COVID-like illness (CLI) case definition on the date of their first SARS-CoV-2 PCR test or during the shedding period; 81 (68.1%) were considered subclinical (did not meet the CLI case definition); 82 (68.9%) individuals were household contacts of index cases. The median duration of viral shedding was 7 days but varied widely from 1 to 29 days.

We identified 101 controls. Eighteen cases did not have sufficient date-matched controls. Using the first enrolled participant (September 1, 2021) as a reference point (19), the median serum collection date for cases and controls were similar (105 versus 117 days). SARS-CoV-2 cases were more frequently female (60.5% vs 48.5%, p=0.08) but were otherwise similar to controls by age and presence of pre-existing medical conditions (**Table 1**). Among cases, there were no differences in demographic characteristics between individuals who shed SARS-CoV-2 for ≤ 7 days (n=45) vs >7 days (n=37) (**Supplemental Table 1**).

To determine if SARS-CoV-2 cases differed from controls in terms of vaccination status, we first confirmed that the duration between vaccination and serum collection between SARS-CoV-2 cases and controls was not significantly different (123 days vs 97 days, $p=0.47$; Table 1). When we compared subgroups, we found that serum samples from vaccinated controls (median 218 days following first enrolled participant) were collected a median 3.7 months later than vaccinated cases (104 days), unvaccinated controls (108 days), and unvaccinated cases (105 days) ($p<0.05$), and thus were more frequently collected during Omicron subvariant circulation (after December 1, 2021; 84.6% vs 63.9%, 56.5%, and 68.7%, respectively; $p=0.018$). Despite this difference in timing of sample collections, there were no overall differences in baseline NAb titer when comparing unvaccinated cases to controls during this time period from January to April, 2022 ($IC_{50}=52.4$ vs 31.3, $p=0.93$, $n=11$).

The proportion of individuals who received ≥ 1 COVID-19 vaccine dose was similar between SARS-CoV-2 cases and controls (46.2% vs 50.5%; $p=0.53$). As outlined in Table 1, cases and controls did have differences in the number of vaccine doses and in hybrid immunity status. Among SARS-CoV-2 cases, after removing 29 individuals who had a COVID-19 vaccine dose between their serum sample collection and their symptomatic visit, these differences persisted (**Supplemental Table 2**).

SARS-CoV-2 cases and controls had similar anti-N IgG positivity rates (76.5% vs 77.2%, $p=0.79$) and anti-N IgG titers (median IgG COI = 13.3 vs 12.8, $p=0.7$; **Figure 2A**). We next measured NAb against the Omicron (BA.1) variant and detected no statistically significant difference in SARS-CoV-2 cases versus controls ($IC_{50} = 28.4$ vs 50.4, $p=0.15$) in the 12 months

preceding their infection (**Table 2, Figure 2A**). Among SARS-CoV-2 cases (n=119), we found no evidence for differences in median Omicron NAb titer between those infected during the 6 months after serum collection (n=72) and those infected 6-12 months after serum collection (n=47) ($IC_{50} = 28.8$ vs 28.4 , $p=0.33$; **Figure 1C**). Symptomatic (CLI) SARS-CoV-2 cases had no significant difference in median IgG (43.2 vs 21.8 , $p=0.33$) or median NAb titer (15.4 vs 10.0 , $p=0.26$) preceding their infection compared to asymptomatic cases.

We evaluated if there were differences in antibody titers based on vaccination status. As outlined in Table 2, among unvaccinated individuals, there was no difference in Omicron NAb titer between cases and controls ($IC_{50}=0.11$). However, among vaccinated individuals (1, 2, or 3 doses), controls had significantly greater NAb titers, including among those considered “up-to-date” on vaccination (155.6 vs 12.1 , $p=0.0001$); anti-N IgG was similar between cases and controls among those considered up-to-date on vaccination ($COI=10.1$ vs 16.3 , $p=0.17$). After removing the 29 cases that had a COVID-19 vaccine dose between their serum sample and their symptomatic visit, these differences persisted (**Supplemental Table 3**).

Among controls, vaccination correlated with enrollment Omicron NAb titers (indicating vaccine- or infection-induced immunity; $R^2=0.20$, $p<0.0001$) but not with anti-nucleocapsid IgG titers (indicating infection-induced immunity; $R^2=0.03$, $p=0.07$) (**Supplemental Figure 2A**). Among unvaccinated individuals, SARS-CoV-2 cases and controls had similar median anti-N IgG ($COI=15.4$ vs 10.0 , $p=0.75$) and Omicron NAb titers ($IC_{50}=38.9$ vs 24.5 , $p=0.11$). Both groups also had similar anti-N IgG positivity rates (79.5% vs 71.4% , $p=0.26$). However, among vaccinated individuals, SARS-CoV-2 cases exhibited 13.3-fold lower Omicron NAb titers than

controls ($IC_{50} = 13.6$ vs 181.2 , $p < 0.0001$) (**Figure 2C**; **Supplemental Figure 1B**). This difference persisted when stratified to individuals based on 1 (11.3-fold, $p = 0.03$), 2 (7.3-fold, $p = 0.20$) and 3 vaccine doses (17.4-fold, $p = 0.0083$) (**Supplemental Figure 2C**).

The median anti-N IgG titer among SARS-CoV-2 cases was 2.4-fold lower than controls (COI = 9.6 vs 23.6), although this difference did not reach statistical significance ($p = 0.12$). We also did not observe a statistical difference in anti-N IgG titers when stratified based on vaccination doses (**Supplemental Figure 2B**). Anti-N IgG positivity rates were significantly lower in vaccinated SARS-CoV-2 cases compared to vaccinated controls (69.4% vs 89.2% , $p = 0.037$; **Figure 3A**). When restricted just to those who received a vaccination within 6 months ($n = 55$), the proportions were similar, though non-significant (74.1% vs 92.9% , $p = 0.06$). There was a significant linear correlation between anti-N IgG and Omicron NAb titers ($R^2 = 0.27$, $p < 0.0001$), due to the contribution of very low and very high-titer anti-N IgG samples (**Figure 3B**).

Finally, we determined if viral shedding duration was linked to Omicron NAb titers. Comparing SARS-CoV-2 cases with a short duration of viral shedding (≤ 7 days) to longer duration (> 7 days), there were no differences in median Omicron NAb titer overall (27.0 vs 54.1 , $n = 0.61$), or stratified by sex ($p = 0.60$) or COVID-19 vaccination ($p = 0.98$).

DISCUSSION

We previously found a high SARS-CoV-2 force of infection in a cohort of Guatemalan agricultural workers (17, 18) and their households (19). Leveraging the same household cohort, we now evaluated the protective role of pre-existing SARS-CoV-2 antibodies (IgG binding and neutralization) against SARS-CoV-2 infection and shedding duration. We found that COVID-19 cases were more likely to have received fewer vaccine doses and were less likely to have preceding hybrid immunity. These findings are consistent with other global studies showing an incremental protective benefit of booster doses and staying up-to-date on vaccination, though the benefit waned quickly over 6 months and with the arrival of new subvariants (33-36). We previously showed rapid waning of NAb titers over 6 months in the AGRI farm worker cohort (18). Future studies should confirm if increased vaccine boosters promoted NAb durability against circulating and future variants in this cohort, as reported in other field sites (37, 38).

Importantly, we showed that both cumulative vaccine doses and recency was associated with higher NAb titers against the circulating strain. Among the 27 SARS-CoV-2 “breakthrough” cases who were considered up-to-date on vaccination yet were still infected, NAb titers were significantly lower than controls (13.1 vs 155.6), suggesting a failure of vaccine to induce an appropriate NAb response among these individuals. This finding has been reported elsewhere (39, 40), and should be explored further in future studies.

Our high nucleocapsid IgG seropositivity (18) and vaccination rate provided an opportunity to study hybrid immunity (41). Indeed, among vaccinated individuals, prior virus infection/exposure was associated with higher levels of NAb against the circulating strain (Omicron) and increased SARS-CoV-2 protection. Zhong *et al* had similar findings, noting in a

pediatric cohort (5-12 years old) from Singapore that NAb titer only correlated with protection against symptomatic COVID-19 in the setting of hybrid immunity and that T cell immunity was the best correlate of protection prior to hybrid immunity (42). However, not all studies consistently support these findings (3-12).

We found that high Omicron NAb titers correlated with nucleocapsid IgG titers (anti-S titers were evaluated in a subset of individuals but were consistently above the limit of detection). Nucleocapsid IgG titers are technically more straightforward to measure than NAb titers, suggesting a potential biomarker for immunity among vaccinated individuals in resource-limited settings. As nucleocapsid is not present in the vaccine, we speculate that this relationship may be due to those with SARS-CoV-2 infections having lower preceding anti-nucleocapsid (infection-induced) immunity. In fact, the high magnitude and durability of NAb responses in the context of hybrid immunity were associated with the induction of memory B cells that in turn was associated with enhanced CD4 T cell responses (43). It would be of interest to understand the T cell responses against diverse viral antigens including nucleocapsid, and the nature of spike epitopes recognized by NAb in this household cohort. Specifically, in-depth monitoring of household contacts in the AGRI-CASA cohort provides a rare opportunity to test the relationship between immune phenotypes and transmission parameters, such as the duration of viral shedding. Here, serum IgG and NAb titers did not predict the duration of viral shedding, with previous studies showing mixed associations, including a potential role for secretory IgA (44-47).

Strengths of our study included a high household enrollment requirement (>75%), frequent saliva sampling irrespective of presence of symptoms, timely surveying and testing of household infections prompted by symptomatic individuals, accurate vaccination records, and a team of experienced, well-trained field and laboratory staff in this rural community. One weakness was that we did not capture SARS-CoV-2 infection history prior to study enrollment, including timing of prior infections, which began in September 2021 when much of the community was already infected. Our SARS-CoV-2 serologic testing occurred up to 12 months prior to infection, which likely resulted in less precise measurements given the known decline in NAb titers over shorter intervals. Although we matched specimens on calendar time, an increased proportion of vaccinated control specimens collected during Omicron circulation could explain relatively higher NAb titers in this group due to exposures; however, there were no difference in NAb titers when we compared unvaccinated cases and controls during this same time period. We also did not evaluate other correlates of protection, such as memory T cell response, which likely play a significant role that may increase over time.

CONCLUSIONS

In a cohort from Guatemala we found that cumulative COVID-19 vaccine doses and greater anti-N IgG and Omicron NAb titer were associated with lower odds of SARS-CoV-2 infection, suggesting a protective role for hybrid immunity. These findings support the importance of continued vaccination efforts in Guatemala, particularly among essential worker populations and their household members who are at high risk of infection and disease. Given a high prevalence of natural infection, and rapid waning of vaccine-induced antibodies, continued vaccination will likely provide the best approach to prevent COVID-19 disease in this population, which we have

383 demonstrated results in significant clinical and economic impacts (48). Nucleocapsid IgG titers
384 may serve as a useful correlate of protection among individuals with hybrid immunity. Future
385 studies should focus on mucosal and systemic longitudinal immunity in the prevention of SARS-
386 CoV-2 infection and disease, including with next generation vaccines.

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Table 1: Comparison of demographics between SARS-CoV-2 cases and matched uninfected non-cases (controls).

Demographic Variable	All subjects	SARS-CoV-2 Cases	Controls	p-value (cases vs controls)
	N = 220	N = 119	N = 101	
Age in years	23.0 (16.5)	22.7 (15.6)	23.3 (17.5)	0.78
Sex = male	99 (45.0%)	47 (39.5%)	52 (51.5%)	0.08
Status				
Index or Co-index	55 (25.0%)	55 (46.2%)	0 (0%)	N/A
Secondary	56 (25.5%)	56 (47.1%)	0 (0%)	
Tertiary	8 (3.6%)	8 (6.7%)	0 (0%)	
Not a case	101 (45.9%)	0 (0%)	101 (100%)	
Pre-existing medical condition	35 (15.9%)	19 (16.0%)	16 (15.8%)	0.98
# vaccine doses at time of enrollment (sample collection date):*				
0	142 (64.55%)	81 (68.1%)	61 (60.4%)	0.07
1	20 (9.09%)	11 (9.24%)	9 (8.9%)	
2	41 (18.64%)	23 (19.33%)	18 (17.82%)	
3	17 (7.73%)	4 (3.36%)	13 (12.87%)	
Received a vaccine dose during the past 12 months (if completed primary series)	58 (26.6%)	27 (22.7%)	31 (30.7%)	0.18
Hybrid Immunity status at sample collection date**				
Unvaccinated/No prior infection	35 (16.0%)	17 (14.3%)	18 (18.0%)	0.08
Vaccinated/No prior infection	15 (6.8%)	11 (9.2%)	4 (4.0%)	
Unvaccinated/Prior Infection	111 (50.7%)	66 (55.5%)	45 (45.0%)	
Vaccinated/Prior infection (hybrid)	58 (26.5%)	25 (21.0%)	33 (33.0%)	

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391 *Cases were enrolled at the time of first confirmed SARS-CoV-2 infection; for controls, we used the same
392 date as their matched cases. Six controls, which were matched based on the date of serum collection, were
393 excluded because they had ended study participation by the time their matched cases developed SARS-
394 CoV-2 infection. **Prior infection was inferred from nucleocapsid IgG positivity and hybrid immunity
395 was defined based on having both documented vaccination and prior infection. Note that one vaccinated
396 control had no nucleocapsid IgG data due to sample availability.

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Table 2: Comparison of SARS-CoV-2 IC50 values preceding SARS-CoV-2 infection (cases) and in matched non-infected controls. (Wilcoxon test by sex and by vaccination status)

Demographic Variable	SARS-CoV-2 Cases N = 119	Controls N = 101	p-value
	Median (IQR)	Median (IQR)	
Overall	28.4 (2.0, 168.2)	50.4 (12.5, 215.9)	0.15
Male sex	19.0 (2.0, 114.2)	43.7 (10.3, 215.9)	0.16
# vaccine doses at enrollment (sample collection date):*			
0	38.9 (9.2, 270.2)	24.5 (2.0, 68.4)	0.11
1	16.1 (2.0, 68.9)	182.7 (45.2, 245.3)	0.01
2	11.9 (2.0, 108.5)	87.0 (31.3, 325.5)	0.04
3	13.4 (7.6, 16.4)	232.5 (179.6, 507.0)	0.004
Considered “up-to-date” at the time of enrollment (sample collection date)	13.1 (2.0, 58.5)	155.6 (79.2, 447.8)	0.0001
Hybrid Immunity status at sample collection date**			
Unvaccinated/No prior infection	2.0 (2.0, 74.0)	2.0 (2.0, 14.2)	0.65
Vaccinated/No prior infection	2.0 (2.0, 11.3)	78.8 (2.0, 185.75)	0.44
Unvaccinated/Prior Infection	59.2 (12.2, 347.0)	41.8 (14.6, 153.7)	0.34
Vaccinated/Prior infection (hybrid)	24.6 (11.9, 91.1)	184.0 (87.4, 491.7)	<0.0001

*Vaccinated prior to the time of serum sample collection. **includes only 4 cases and 13 controls

Figure 1

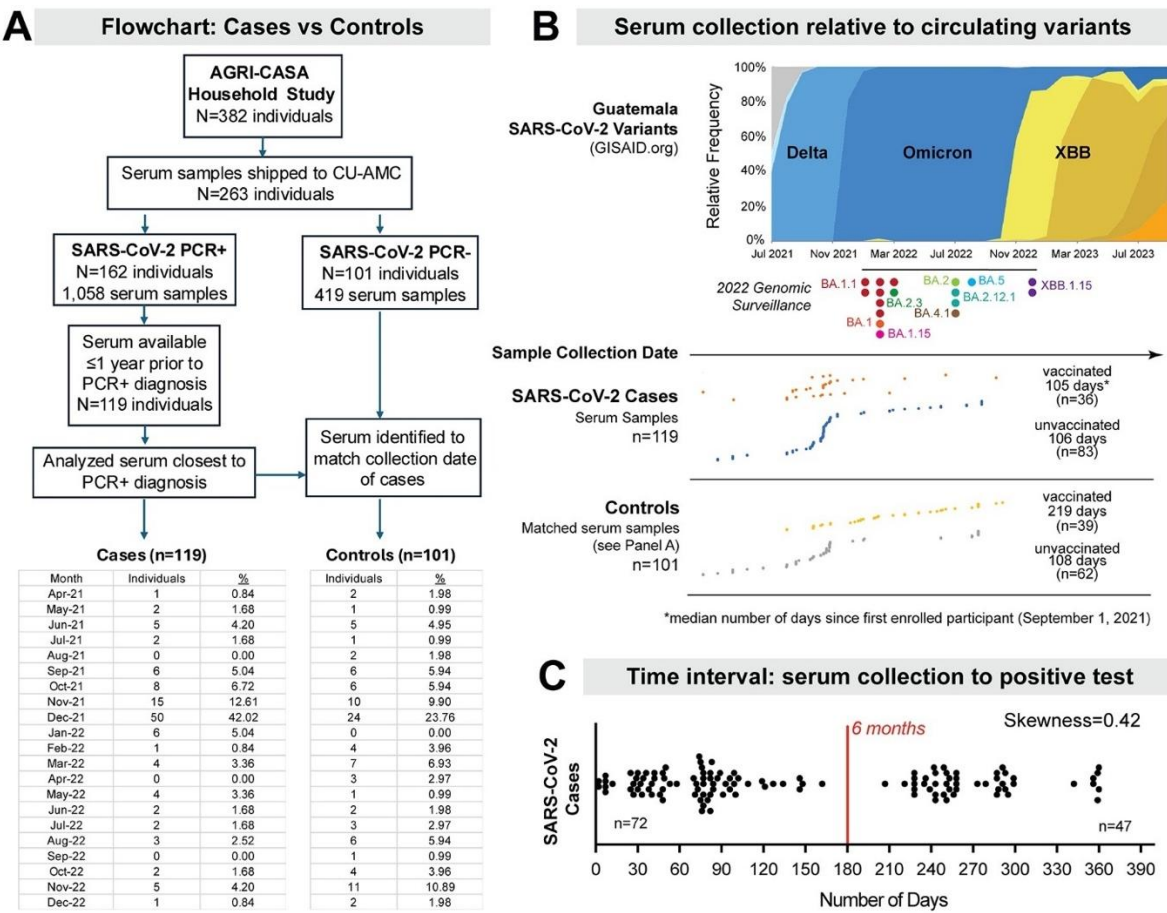


Figure 1: Evaluation of Pre-Existing Serological Immunity in AGRI-CASA. (A) Flowchart for selection of SARS-CoV-2 cases and controls. Serum samples from the 2 cohorts were matched for collection date to capture similar circulating variants. (B) Circulating variants in Guatemala relative to date of serum sample collection. Each dot corresponds to serum samples from SARS-CoV-2 cases and month-matched controls, subdivided based on vaccination status. Each dot in the Genomic Surveillance inset corresponds to a full-length SARS-CoV-2 sequence from one individual collected during specific months in 2022. Sampling occurred primarily during waves due to Omicron (BA.1) and its subvariants based on deposited SARS-CoV-2 sequences from AGRI-CASA and sequences from Guatemala deposited in GISAID.org. The median number of days for sample collection of each group was noted, relative to the first reported subject enrolled (Sep 1, 2021), showing vaccinated controls were sampled about 3.7 months later than the other groups. (C) Distribution of time intervals between sample collection and positive SARS-CoV-2 test for cases, showing a bimodal distribution (skewness=0.42).

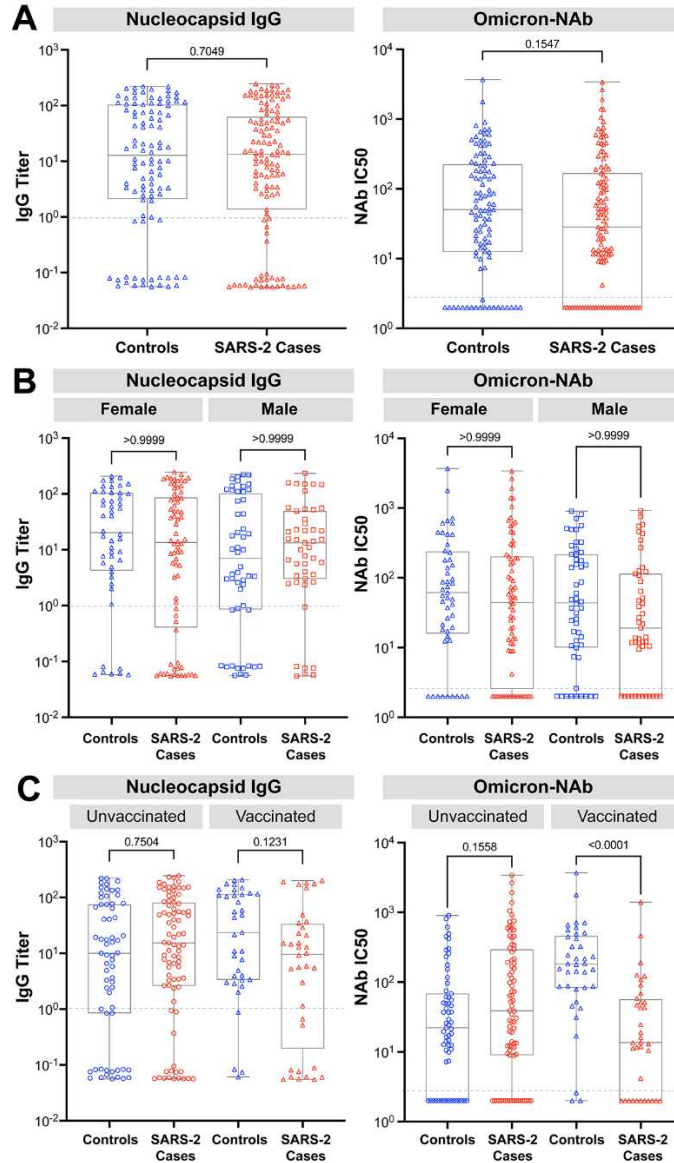
420 **Figure 2**

Figure 2: Antibody titers in serum samples prior to documented SARS-CoV-2 infection in AGRI-CASA. Serum samples were evaluated for nucleocapsid (N) IgG titers (left panels) and Omicron neutralizing antibody (NAb) titers (right panels). Each dot corresponds to a single individual, analyzed (A) without stratification, and (B,C) stratified according to (B) Sex and (C) Vaccination status. Bars and lines correspond to median $\pm$ IQR. Data were analyzed using One-Way ANOVA (Kruskal-Wallis test) followed by a Dunn's Multiple Comparisons test, with p-values indicated.

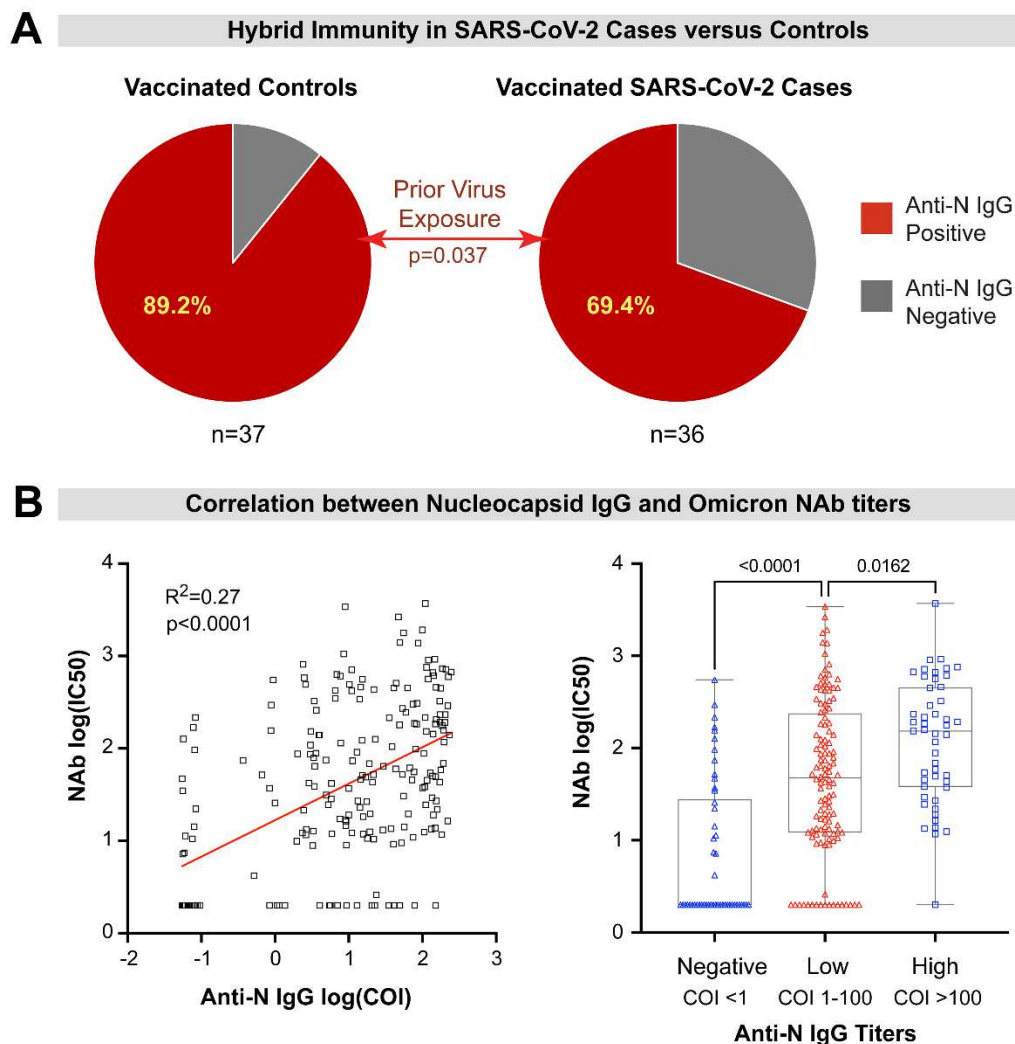
429 **Figure 3**

Figure 3. Evaluation of Hybrid Immunity in the AGRI-CASA study. Individuals who were previously vaccinated were evaluated for anti-N positivity, which is an indicator of prior virus exposure. (A) The proportion of anti-N IgG+ individuals were compared between vaccinated controls and SARS-CoV-2 cases by Chi-Square test. (B) Linear correlation between log-transformed anti-N IgG and Omicron NAb titers for the entire cohort which included both vaccinated and unvaccinated individuals (left panel). Omicron NAb titers were compared between samples with negative, low and high anti-N IgG titers. Median $\pm$ IQR values are shown and evaluated using the Kruskal-Wallis test and Dunn's Multiple Comparison post-hoc test, with p-values shown.

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