Seroprevalence of anti-SARS-CoV-2 IgG and IgA antibodies before the launch of COVID-19 vaccination in Kazakhstan.

ClinicalTrials.gov #NCT04871841

Abstract
Background: COVID-19 exposure in Central Asia appears underestimated and SARS-CoV-2 seroprevalence data are urgently needed to inform ongoing vaccination efforts and other strategies to mitigate the regional pandemic. Here, we assessed the prevalence of anti-SARS-CoV-2 antibody-mediated immunity in a heterogeneous cohort of public university employees in Karaganda, Kazakhstan.

Methods: Asymptomatic subjects (n=100) were randomly recruited prior to their first COVID-19 vaccination. Questionnaires were administered to capture a range of demographic and clinical characteristics. Nasopharyngeal swabs were collected for SARS-CoV-2 RT-qPCR testing. Serological assays were performed to detect spike (S)-reactive IgG and IgA.

Results: Anti-S IgG and IgA seropositivity rates among SARS-CoV-2 PCR-negative participants (n=100) were 42% and 53%, respectively, and 61% of subjects tested positive for at least one of the antibodies. Serologically confirmed history of COVID-19 was associated with self-reported history of respiratory illness since March 2020 (p<0.001).

Conclusions: SARS-CoV-2 exposure in this cohort is ~15-fold higher compared to the reported all-time national and regional COVID-19 prevalence. Continuous serological surveillance is critical for understanding the COVID-19 transmission dynamics and should be nationally implemented to better inform the public health response in Central Asia.

AUTHOR APPROVAL
All authors indicate that have seen and approved the manuscript.

DECLARATION OF INTERESTS.
The authors declare that they have no competing interests.

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INTRODUCTION.
COVID-19 remains a global public health concern and is especially pernicious in regions with limited public health infrastructure that suffer from inadequate epidemiologic surveillance and delayed implementation of pandemic countermeasures. In the Central Asian states, such as Kazakhstan, substantial underestimations (of ~14-fold) of COVID-19 incidence and associated mortality \(^1\)-\(^3\) have led to public distrust and slow uptake of public health measures, including vaccination \(^4\). To date, the extent of community exposure to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in Kazakhstan remains unclear. Thus, as of 7 August 2021, the officially reported number of all-time COVID-19 cases in Kazakhstan was 689,402 (626,402 of which were PCR-confirmed), representing a cumulative prevalence of ~3.7% \(^5\)- a figure that appears low given the substantial excess of infections and mortality consistent with COVID-19 \(^2,3\).

Disparities between reported cases and true infections occur due to a plethora of factors, including unreported asymptomatic and mild infections, limited access to timely clinical and laboratory confirmation of COVID-19 diagnosis, and false-negative laboratory test results \(^6\). One way to estimate the proportion of the population with previous exposure to COVID-19 is by using serological surveillance \(^6\), which has been under-utilized in Kazakhstan and other Central Asian states.

Here, to gain insight into the true SARS-CoV-2 exposure rates in Kazakhstan, we assessed full-length SARS-CoV-2 Spike(S)-specific IgG and IgA titres in a public university-based cohort, consisting of instructors, administrative and laboratory staff, and healthcare practitioners - representing a diverse array of people with different risks of exposure to COVID-19.

METHODS.
Study setting and participant recruitment. This study was conducted in conjunction with screening for a clinical trial assessing immunogenicity of the Sputnik-V vaccine (ClinicalTrials.gov #NCT04871841) based in Karaganda, the capital of Karaganda region situated in Central Kazakhstan. Since February 2020, the Karaganda region (population ~1.3M) has had over 63,000 (~5% of regional population) reported COVID-19 infections, placing it behind several other locales including the capital, Nur-Sultan (population ~ 1.0M), which has had a reported all-time COVID-19 prevalence of >11%⁷. Participant screening occurred in April-May 2021 at a COVID-19 vaccination clinic for university employees at the Karaganda Medical University. Consenting, asymptomatic adults, who had not previously received a COVID-19 vaccine, were invited to participate in the study. Exclusion criteria were presence of respiratory symptoms or laboratory-confirmed COVID-19 diagnosis within two weeks prior to the study. Short questionnaires addressing the participants' demographic background and recent history of COVID-19 exposure were administered.

Sample collection and processing. Nasopharyngeal swabs were collected following the national guidelines into DNA/RNA shield media (Zymo Research, Irvine, US). Blood (5 ml) was collected by venipuncture into EDTA tubes (Improvacuter, Gel & EDTA.K2, Improve Medical Instruments, Guangzhou, China). Blood plasma was isolated by centrifugation at 2,000 × g for 10 minutes. All samples were stored at -80 °C prior to analyses.

PCR screening for SARS-CoV-2. Total RNA was isolated from nasopharyngeal swabs by magnetic bead-based nucleic acid extraction (RealBest Sorbitus, Vector-Best, Novosibirsk, Russia) and used for SARS-CoV-2 real-time RT-PCR testing by the Real-Best RNA SARS-CoV-2 kit (Vector-Best, Novosibirsk, Russia) targeting the SARS-CoV-2 RdRp and N loci, following the manufacturer's protocol.
IgG and IgA assays. Anti-SARS-CoV-2 S1 IgG and IgA ELISAs were performed using commercially available assays (Euroimmun Medizinische Labordiagnostika AG, Lübeck, Germany) on the Evolis 100 ELISA reader (Bio-Rad) according to the manufacturers’ protocols. Optical density (OD) ratios were calculated as ratio of the OD reading for each sample to the reading of the kit calibrator at 450 nm. In the initial analysis, we used the Euroimmun-recommended OD ratio cutoff values for both IgG and IgA, which are “<0.8” for Ig-negative samples, “0.8-1.1” for Ig-borderline samples, and “>=1.1” for Ig-positive samples.

We noted that using the manufacturer's cutoff values: of all IgG "borderline" participants (n=9), 7 (77.8%) were IgA+ (IgA OD ratio>=1.1), 1 (11.1%) was IgA borderline and 1 (11.1%) was IgA negative (Fig 1), while of all IgA "borderline" participants (n=6), 2 (30.0%) were IgG+ (IgG OD ratio>=1.1), 1 (20.0%) was IgG borderline, and 3 (50.0%) were IgG negative. Since most IgG "borderline" samples were IgA+, we reasoned that setting the threshold for IgG positivity at “0.8” would allow to simplify the participant serostatus classification while still capturing most SARS-CoV-2-exposed subjects. For IgA, we kept the positivity threshold at the manufacturer-recommended value (1.1) and considered all samples with IgA OD ratios <1.1 “IgA-negative”.

The higher cutoff threshold for the Euroimmun IgA compared to IgG is consistent with earlier research 8. Thus, using the in-house thresholds, we defined the "no prior COVID-19" subjects as negative for both IgG and IgA (IgG-, IgA-) and the "prior COVID-19" subjects as positive for either or both IgG and/or IgA (IgG+/-, IgA+/-).

Statistical analysis

We used the two-sided Mann-Whitney U, Pearson $\chi^2$, or Fisher's exact tests to compare differences between groups, as appropriate. Correlations among variables were explored using the Spearman rank test.
Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. All authors had full access to all the data in the study and the lead authors (IK, SY, DB) had final responsibility for the decision to submit manuscript for publication.

RESULTS.

All 100 participants tested negative for SARS-CoV-2 by RT-qPCR at screening. Of 100 participants, 42 (42%) were IgG+. Due to insufficient blood samples, we were unable to include two samples (one IgG+ and one IgG-) in IgA testing, thus out of 98 IgA-tested participants, 52 (53.1%) were IgA+ (Fig 1a). When stratified by the presence/absence of both IgG and IgA, there were 34 (34.7%) IgG+/IgA+, 7 (7.1%) IgG+/IgA-, 18 (18.4%) IgG-/IgA+ and 39 (39.8%) IgG-/IgA- subjects (Fig 1b). Cumulatively there were 60.6% (60/99) subjects positive for at least one of the antibodies; these subjects were defined as the "prior COVID-19" group (Table 1). IgG and IgA OD450 ratios correlated strongly across the cohort (r=0.599, p<0.001, Fig 1b).

There were no significant clinical or demographic differences between the "prior COVID-19" and "no prior COVID-19" groups. We did note, however, that the "prior COVID-19" group consisted of almost 20% more people self-identifying as ethnic Kazakhs, compared to the "no prior COVID-19" group (Table 1). The "prior COVID-19" subjects more frequently self-reported having had a respiratory illness since March 2020 (p<0.001, Table 1), which only in a minority of cases (24%, 8/33) was confirmed as COVID-19 by PCR and/or serology; most of the self-reported respiratory illness occurred in March-Aug 2020, and in two cases in February-March 2021.
DISCUSSION.

Here we present anti-S IgG and IgA-based seroprevalence findings from a cohort of public university employees in Kazakhstan, who were invited to participate in the study prior to receiving their first dose of COVID-19 vaccine. The cohort seropositivity for anti-S IgG and IgA was 42% and 53%, respectively, while over 60% of the subjects tested positive for at least one of the antibodies. Consistent with studies of excess infection and death \(^2,3\), the serologically assessed SARS-CoV-2 exposure in this cohort was 14-15-fold higher than the reported all-time national and regional COVID-19 prevalence.

The substantial discrepancy between serology-derived and officially reported COVID-19 exposure estimates is not uncommon across the globe \(^6\). However, what is most striking about our findings is the unusually high SARS-CoV-2 seroprevalence exceeding the estimates for many other countries \(^6\), albeit on par with the recent estimates from the neighbouring St. Petersburg, Russia, where antibodies against the receptor binding domain of the SARS-CoV-2 spike were detectable in \(\sim 45\%\) of randomly sampled adults \(^9\). Similarly high SARS-CoV-2 seroprevalence rates have also been observed in the general populations of Ecuador \((\sim 45\%\) \(^10\)), India and Pakistan \((>52\%\) \(^11,12\)), and in communities at risk, such as healthcare workers and nursing home residents, across the globe \(^6\).

In our cohort, serologically confirmed exposure to COVID-19 was associated with self-documented history of respiratory illness, most of which was dated by the participants to the peak of the first COVID-19 wave in the Spring-Summer of 2020 \(^1\), period during which the country's healthcare system was overwhelmed and laboratory testing was limited \(^3\). This timing of self-reported illness suggests that anti-S immunoglobulins remain detectable up to a year after
symptomatic COVID-19, consistent with the established long-term persistence of SARS-CoV-2-reactive antibodies. Somewhat unexpectedly, we did not see any association between the serologically confirmed exposure to COVID-19 and the participants' professional occupation or any demographic factors, although there were proportionally more ethnic Kazakh subjects among seropositive participants. This may be because differences between sub-populations with different COVID-19 exposure risk are overwhelmed by the high seroprevalence in the general population. Alternatively, the risk of infection for healthcare workers may not be elevated relative to the general population because of the adequacy of infection control practices that are in place in healthcare facilities.

Given the logistical difficulties with procuring biomedical reagents and limited technological capacity in the setting of Kazakhstan, our choice of the serologic assay in this study was dictated by both assay quality and logistic feasibility. Therefore, we used a commercially available, FDA-approved assay, validated by several research groups, and deployable in a basic clinical lab setting. Furthermore, we chose to use both IgG and IgA based on the evidence of distinct but overlapping temporal patterns seen for these antibodies in COVID-19 patients. Thus, both IgG and IgA appear as early as 2 weeks post-symptom onset (PSO), with IgA increasing up to third week PSO and then dropping, while IgG increases until fourth week PSO, remaining detectable up to 8 months PSO. Finally, we chose not to use IgM, since this antibody is more suitable for detecting acute infection, while in convalescent subjects IgA temporally overlaps IgM, resulting in higher positivity rates.

Recent studies indicate that people with prior history of COVID-19 have a stronger response to vaccination compared to COVID-19-naive subjects after one vaccine dose. Mass COVID-19 vaccination was launched in Kazakhstan in February 2021, and so far, ~30% of Kazakhstan’s
population has received at least one vaccine dose \(^7\). Considering limited vaccine supply and low vaccination acceptance, our seroprevalence findings therefore could be extended to inform the ongoing vaccination efforts about the existing population-wide anti-SARS-CoV-2 immunity in Kazakhstan. For example, the second dose of COVID-19 immunisation could be reserved for people without prior natural exposure to SARS-CoV-2 and delayed for subjects with prior COVID-19 exposure.

Our study has limitations. First, given the small sample constrained to employees of one, albeit large (employing ~3000 staff) organization, our findings should be seen as preliminary “pilot” data on SARS-CoV-2 prevalence in Kazakhstan. Next, our analysis did not include a thorough IgG/IgA testing of control (pre-pandemic and acute infection) samples, and it is possible, although highly unlikely, that the assay performance is affected by factors such as cross-reactivity with infections endemic to Kazakhstan. Future studies will need to assess the performance of these and other serologic assays across various demographic groups in the country.

**Conclusions.**

Continuous epidemiologic surveillance of SARS-CoV-2 exposure is critical for understanding the COVID-19 transmission dynamics and for informing ongoing vaccination efforts and other COVID-19 mitigation strategies. Although constrained by a small sample size, our seroprevalence study for the first time documents an extremely high rate of SARS-CoV-2 exposure in Kazakhstan. These findings should pave way to larger seroprevalence surveys accounting for, among other factors, vaccine-induced immunity.

**CONTRIBUTORS.**
Conceptualization, IK, SY, DB. Investigation and formal analysis, IK, SY, BN, YK, SK, IIK, DV, MSM, GH. Clinical and laboratory site supervision IK, LA, AT. Writing – original draft, IK, SY. Writing – review and editing, IK, SY, BN, YK, SK, IIK, LA, AT, M.S.M., GH, DB. Funding acquisition, IK, GH, DB.

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LIST OF FIGURES

**Figure 1:** a) Distribution of optic density (OD) 450 ratios for Spike-reactive IgG and IgA among study participants. Blue dotted lines represent the manufacturer-recommended assay cut-off values at OD450 ratios 0.8 and 1.1. b) Correlation plot of IgG and IgA OD450 ratios among study participants. Blue dotted lines represent the assay cut-off values at OD450 ratios 0.8 and 1.1, for IgG and IgA, respectively, used in this study for participant classification by serostatus.

LIST OF TABLES

**Table 1:** Demographic and clinical characteristics of the study cohort.

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Fig 1

(a) IgG OD450 Ratio

(b) IgA OD450 Ratio

IgG-/IgA+ 18%
IgG+/IgA+ 35%
IgG-/IgA- 40%
IgG+/IgA- 7%