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Original Research Article

Nationwide Seroprevalence of SARS-CoV-2 in Saudi Arabia

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ABSTRACT

Background: Estimated seroprevalence of Coronavirus Infectious Disease 2019 (COVID-19), caused by the Severe Acute Respiratory Syndrome-Coronavirus-2 (SARS-CoV-2) is a critical evidence for a better evaluation of the virus spread and monitoring the progress of COVID-19 pandemic in a population. In the Kingdom of Saudi Arabia (KSA), SARS-CoV-2 seroprevalence has been reported in specific regions, but an extensive nationwide study has not been reported. Here, we report a nationwide study to determine the prevalence of SARS-CoV-2 in the population of KSA during the pandemic, using serum samples from healthy blood donors, non-COVID patients and healthcare workers (HCWs) in six different regions of the kingdom, with addition samples from COVID-19 patients.

Methods: A total of 11,703 serum samples were collected from different regions of the KSA including; 5395 samples from residual healthy blood donors (D); 5877 samples from non-COVID patients collected through residual sera at clinical biochemistry labs from non-COVID patients (P); and 400 samples from consented HCWs. To determine the seroprevalence of SARS-CoV-2, all serum samples, in addition to positive control sera from RT-PCR confirmed COVID-19 patients, were subjected to in-house ELISA with a sample pooling strategy, which was further validated by testing individual samples that make up some of the pools, with a statistical estimation method to report seroprevalence estimates.

Results: Overall (combining D and P groups) seroprevalence estimate was around 11% in Saudi Arabia; and was 5.1% (Riyadh), 1.5% (Jazan), 18.4% (Qassim), 20.8% (Hail), 14.7% (ER; Alahsa), and 18.8% in Makkah. Makkah samples were only D group and had a rate of 24.4% and 12.8% in the cities of Makkah and Jeddah, respectively. The seroprevalence in Saudi Arabia across the sampled areas would be 12 times the reported COVID-19 infection rate. Among HCWs, 7.5% (4.95–10.16 CI 95%) had reactive antibodies to SARS-CoV-2 without reporting any previously confirmed infection. This was higher in HCWs with hypertension. The study also presents the demographics and prevalence of co-morbidities in HCWs and subset of non-COVID-19 population.

Interpretation: Our study estimates the overall national serological prevalence of COVID-19 in Saudi Arabia to be 11%, with an apparent disparity between regions. This indicates the prevalence of asymptomatic or mild unreported COVID-19 cases.

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Introduction

The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is a novel coronavirus that was first identified in Wuhan city in China by the end of 2019 and spread globally to cause epidemics in almost all parts of the world. In March 2020, the World Health Organization declared it a global pandemic. The disease, named coronavirus disease 2019 (COVID-19), causes a respiratory illness that could range from severe pneumonia to mild respiratory illness with symptoms such as fever, dry cough, fatigue, headaches, shortness of breath and gastroenteritis (diarrhea). However, some cases are completely asymptomatic [1]. As of January 2021, there have been more than 90 million confirmed cases with ~1.9 million deaths worldwide [1].

While immune responses against SARS-CoV-2 could be induced as early as the first week after symptoms onset, seroconversion usually occurs at a median of 10–12 days for IgM and 12–15 days for IgG and could reach 100% by 21 days in most infected individuals [2–4]. Serum IgM levels peak at two to three weeks, whereas the IgG antibodies peak at three to four weeks post symptoms [4]. This increase of antibodies over time is usually accompanied by decreased viral RNA in the respiratory tract [3]. The neutralizing activity of these antibodies, particularly IgG, peaks at one month and could last for up to eight months post symptoms onset [5]. Nonetheless, some reports suggest a decline in the levels of neutralizing antibodies (NAb) after three months post-infection [6].

Seroprevalence studies on COVID-19 are being conducted in many countries to estimate the true prevalence of COVID-19; especially that COVID-19 can be asymptomatic and are not reported by clinical investigation [7–9]. These studies aid in defining the disease burden, gauging the need for vaccine coverage, establishing the correlates of protection, evaluating the possibility and impact of re-infections, and evaluating the benefits of public health measures such as lockdown and travel bans. Several studies showed that the seroprevalence of COVID-19 in general populations varies across the world [10–13]; however, there is still a need for conducting more seroprevalence studies to provide information considering the variability of specific populations, time of sampling, locations, and type of the measured immune responses.

Therefore, we here present a nationwide COVID-19 serological prevalence study in the Kingdom of Saudi Arabia (KSA) with serum samples collected from four different populations in six provinces of the country. The populations are healthy blood donors (D), non-COVID-19 patients (P), confirmed COVID-19 patients, and HCWs. The sampled regions are the provinces of Riyadh (RYD), Jazan (JZN), Qassim (QSM), Hail, Eastern Region (ER – Alahsa governorate area), and Makkah. The latter was divided into Makkah City (MKH) and Jeddah City (JDH) in order to highlight the burden of COVID-19 and seroprevalence in the holy city of Makkah since it is a travel destination for millions of pilgrims from across the globe and would support more efficient global health measures. This study present estimated seroprevalence based on an in-house ELISA with a sample pooling strategy, which was further validated by testing individual samples that make up some of the pools, with a statistical estimation method. This study also presents the chronic diseases and demographics of a subset of non-COVID-19 patients and HCWs.

Materials and methods

Samples and subjects

In this seroprevalence cross-sectional study, a total of 11,703 serum samples were collected from different regions including 5395 residual samples from healthy blood donors (D); 5877 samples from residual sera at clinical biochemistry labs from non-COVID patients (P); 31 samples from consented infected COVID-19 patients within ten days of infection; and 400 samples from consented HCWs.

Samples collected from D and P were from six Saudi Arabian provinces (regions): Riyadh (RYD), Jazan (JZN), Qassim (QSM), Hail, Eastern Region (ER, Alahsa governorate in particular), and Makkah Region in which samples were collected from the cities of Makkah (MKH) and Jeddah (JDH). From the following hospitals: National Guard Health Affairs (NGHA) hospitals in Riyadh and Alahsa, Jazan University Hospital in Jazan, Central Blood Bank in Qassim, Central Blood Bank in Hail, King Abdullah Medical Complex in Jeddah and Central Blood Bank in Makkah. Samples from HCWs and COVID-19 patients were collected from Prince Mohammad bin Abdulaziz hospital and NGHA hospital in Riyadh city. The non-COVID-19 patients were defined as any person who is visiting a clinic or admitted at a hospital with samples collected for clinical biochemistry blood testing and confirmed to be negative for COVID-19 by RT-PCR. Time of sample collection: samples were collected from Jun to Aug, except for Alahsa, Makkah, and Jeddah where sampling was done throughout November (Table 1).

ELISA

An in-house enzyme-linked immunosorbent assay (ELISA) was developed and applied to detect IgG against SARS-CoV-2 in serum samples, following previously published protocols [14,15]. Nunc MaxiSorp 96-well ELISA microplates (Thermo Fisher, Waltham, MA) were coated with recombinant S1 subunit of the SARS-CoV-2 Spike protein (Sino Biological, China) at a concentration of 1 μg/mL. The plates were incubated overnight at room temperature (RT). Plates were then washed six times with washing buffer (phosphate buffered saline (PBS) with 0.5% Tween20, PBS-T) using automated Microplate Washer (Molecular Devices, San Jose, CA). Then, wells were blocked by washing buffer containing 10% skimmed milk (blocking buffer) for one hour at RT. Serum samples from COVID-19 patients and HCWs were diluted at 1:100 in PBS-T, and 50 μl of each diluted sample were added into duplicate wells and incubated for two hours. Samples from blood donors and non-covid-19 patient were prepared in pools of 10 samples by adding 2 μl from each serum sample to 180 μl of PBS-T (i.e. 1:100 dilution), and 50 μl of each diluted pool were added into duplicate wells and incubated for two hours. Some positive pooled samples were subsequently tested as individual samples for confirmation. Plates were washed, and 50 μl of 1:1000 diluted alkaline phosphatase labeled goat anti-human IgG secondary antibody (Thermo Fisher, Waltham, MA) were added and incubated for one hour at RT. After washing, a substrate made of PNPP (pnitrophenylphosphate, sigma) tablets dissolved in diethanolamine buffer solution and distal water was added. This was followed by measuring optical density (OD) at 405 nm using Microplate Reader (Molecular Devices, San Jose, CA). The positive control samples were collected from two confirmed COVID-19 recovered cases and the negative control samples were collected prior to COVID-19 pandemic. The cut-off value was set as the average of the negative control serum samples plus three times of standard deviation. Negative control samples were sera collected before the COVID-19 pandemic, and positive control samples were from confirmed recovered COVID-19 cases. The same control samples were aliquoted and used in every ELISA run. ELISA was repeated on different pools from all regions in order to confirm positivity.

Confirmatory ELISA

Two confirmatory ELISA tests were performed in order to confirm the pooled sample results and assess the average number of

Table 1
Estimated seroprevalence rate of COVID-19 in six Saudi Arabian regions based on blood donors and non-COVID-19 patients.

Region	Population size	COVID-19 incident rate (%)	No of samples	Sample collection time	Seroprevalence (%)	Seroprevalence 95% CI
Sampled regions	24,750,893	1.06	11,275	Jun–Nov 2020	10.9	(10.3, 11.6)
Blood donors			5385		8.8	(8, 9.7)
Non-COVID-19 patients			5890		13.3	(12.4, 14.2)
Riyadh	8,014,678	0.881	4237	20-Jun	5.1	(4.5, 0.8)
Blood donors			1490		2.4	(1.5, 3.2)
Non-COVID-19 patients			2747		6.3	(5.5, 0.1)
Jazan	1,535,167	0.78	640	20-Jun	1.6	(0.7, 2.5)
Blood donors			80		0	(0, 0)
Non-COVID-19 patients			560		1.8	(0.8, 2.8)
Qassim	1,389,929	0.92	3032	Jul–Aug 2020	18.4	(17, 19.8)
Blood donors			1632		13.7	(11.8, 15.6)
Non-COVID-19 patients			1400		24.5	(22.8, 26.2)
Hail	685,423	0.98	460	Jul–Aug 2020	20.9	(18.3, 23.5)
Blood donors			300		8	(6.5, 9.5)
Non-COVID-19 patients			160		42.2	(36.7, 47.7)
Alahsa	4,787,375	1.8	1874	20-Nov	14.7	(12.7, 16.6)
Blood donors			851		10.6	(7.6, 13.6)
Non-COVID-19 patients			1023		16.8	(14.7, 19)
Makkah	8,338,321	1	1032	20-Nov	18.8	(15.8,21.9)
Blood donors Makkah City			532		24.5	(22.3, 26.6)
Blood donors Jeddah City			500		12.9	(9,16.8)

positive samples per pool. First, individual samples from 19 pools (n = 190) were evaluated using anti-SARS-CoV-2 ELISA IgA (EUROIMMUN, Germany) following the manufacturer's instruction. ELISA ratios below 0.8 and above 1.1 were considered negative and positive, respectively, as per the manufactures' instructions. Second, individual samples were selected from pools that have a range of higher and lower OD values: samples from 57 pools (n = 570), ten pools from each region (5 from each group; donors and non-COVID-19 patients) were evaluated using the in-house anti-SARS-CoV-2 spike IgG ELISA as described above.

Statistical analysis

Samples pooling strategy has been recommended to expand test coverage and save resources by eliminating the number of tests required for diagnostic, screening, and surveillance programs [16,17]. Our estimates are derived based on 10 samples per pool. Thus, a positive pool includes at least one (10%) positive sample. Stratified by region and population, we obtained the average number of positive samples in positive pools. Then, we randomly selected five positive pools per population from all regions, except for Jazan D population, for individual testing to obtain the average number of positive individual samples per population per region. Therefore, the prevalence for each region and population were obtained based on the equation below with 95% confidence intervals obtained using the pool's standard error of the mean.

$$\frac{\#postiviepools}{\#pools} \times 0.1 \times averagenumberofpositiveindividualsamples$$

Demographic information were summarized using descriptive statistics in terms of mean and standard deviation or frequencies and percentages. For the chronic diseases in the HCWs in Riyadh, group comparisons were made based on chi-square tests, and the level of significance is alpha = 0.5. All statistical analyses were conducted using SAS 9.4 (SAS Institute Inc., Cary, NC) and GraphPad Prism V8 software (GraphPad Co.).

Results

Demographics and chronic diseases of the study subjects

The blood donor population (group D) was predominantly male with an average age ranging from 26 to 32 in the sampled regions; the non-COVID-19 patient population (group P) was balanced in terms of gender distribution in most regions with an age range of 24–58 in different regions (Table S1). Among the P group (n = 5877), 3592 subjects sampled in Eastern Region (Alahsa) and Riyadh region were further analyzed to highlight the presence of chronic diseases, which includes hypertension, diabetes, asthma, and dyslipidemia. The prevalence of chronic diseases in this subset population of non-COVID-19 patients were 51.7% obesity (BMI > 40), 45% diabetes, 49% hyperlipidemia, 35% hypertension, and 3.3% asthma. No formal association was done with the seroprevalence due to the nature of pooling sera for testing.

National seroprevalence of SARS-CoV-2 in Saudi Arabia

To estimate the COVID-19 seroprevalence in KSA, a total of 11,272 samples were collected from six provinces of KSA; 5395 samples were collected from the blood donors and 5877 samples from the non-COVID-19 patients (Table 1). These sera were tested in pools of 10 samples, and the results showed high seropositivity in pooled samples compared to negative and positive quality control samples (Fig. 1 A and B). Individual serum samples from 57 selected positive pools (10 pools per region; 5 from group D and 5 from group P, except 2 pools from Jazan blood donors since they were negative) were tested for anti-SARS-CoV-2 spike IgG (Fig. 1C). Further, individual serum samples from 19 selected positive pools were tested for anti-spike IgA ELISA (Fig. 1D).

The average number of positive individual samples per pool was used to calculate the seroprevalence per population and per region. Regional seroprevalence in the D group was 2.36% in Riyadh, 0% in Jazan, 13.65% in Qassim, 8% in Hail, 10.62 in Eastern Region (Alahsa areas), and 18.84% in Makkah (24.45% and 12.88 in the cities of Makkah and Jeddah, respectively). Regional seroprevalence in the P group was 6.30% in Riyadh, 1.78% in Jazan, 24.49% in Qassim, 42.18% in Hail, and 16.84 in Eastern Region (Alahsa areas). There was a

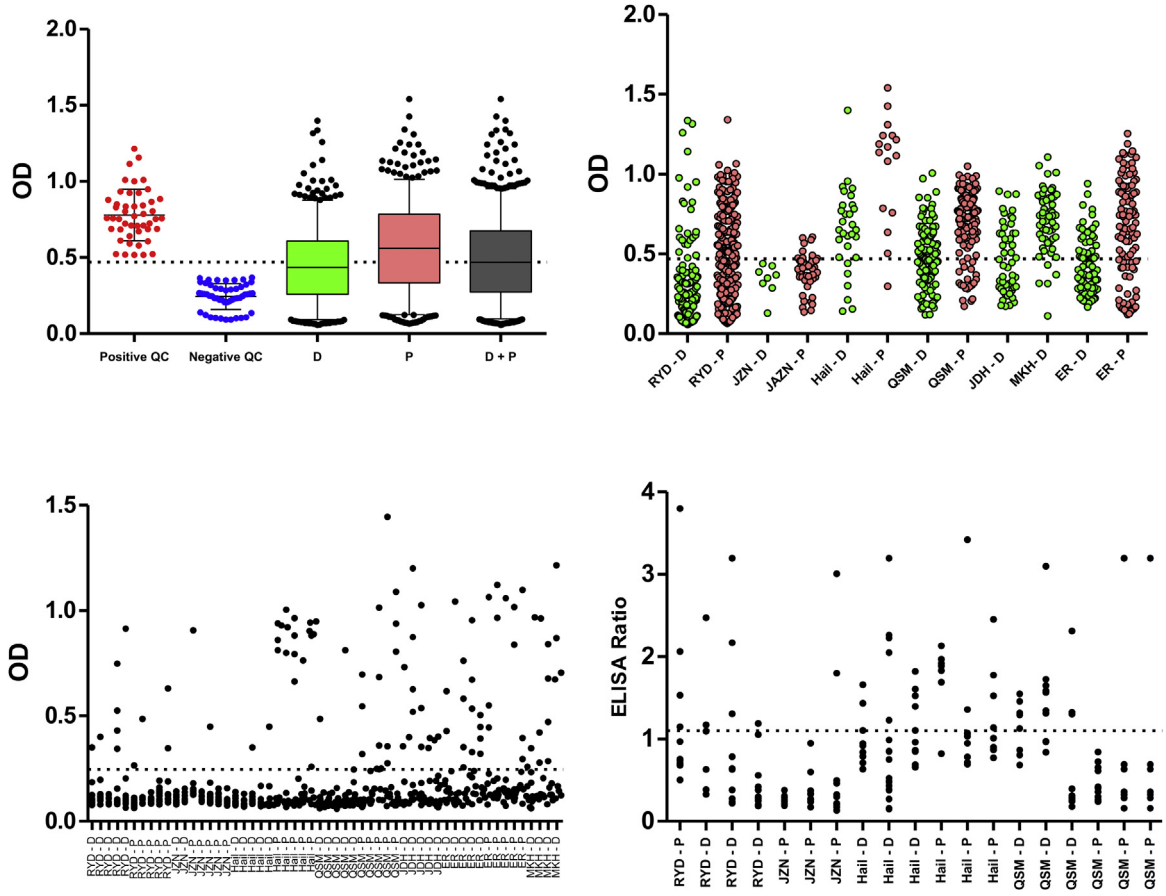

Fig. 1. Anti-SARS-CoV-2 antibodies in serum samples from Saudi Arabia.
In-house ELISA for anti-SARS-CoV-2 spike IgG antibodies (A, B, and C). **A:** Serum samples from blood donors (D; green) and non-COVID-19 patients (P; red) were pooled (10 samples per pool) and evaluated along with positive and negative quality control (QC) samples. The same control samples were tested in quadruplicates in each ELISA plate and values from different plates are plotted. **B:** Pooled samples (10 per pool) from D (green) and P (red) are plotted for each region. Regions' names were included as described in the text. Individual samples that made up some of the pools were further tested using in-house anti-spike IgG ELISA (**C**) or using commercial anti-spike IgA ELISA (**D**). The in-house ELISA's cut-off value was calculated as the average of negative controls plus three times the standard deviation. The cut-off value for commercial ELISA was 1.1. QC: Quality control samples.

trend of higher seroprevalence estimate in P group as compared to D group (Fig. 1A). The overall regional seroprevalence estimate (combining D and P groups) was 11% in Saudi Arabia; and was 5% in Riyadh, 1.5% in Jazan, 18% in Qassim, 20.8% in Hail, and 14.7% in Alahsa. The seroprevalence estimates were compared to the COVID-19 incident (infection) rate in December 2020, in the sampled regions, showing a trend of increasing seroprevalence higher COVID-19 incident rate (sampled regions in Table 1 and Fig. 2; unsampled regions in Table S2). This indicates that the seroprevalence rate is more than the incident rate by around 6x (Riyadh), 2x (Jazan), 20x (Qassim), 21x (Hail), 8x (Alahsa), and 18x (Makkah), were x means how many times. Based on this, the seroprevalence in Saudi Arabia across the sampled regions would be 12x more than the incident rate (Table 1 and Fig. 2). However, the fold difference between seroprevalence and incident rates is not consistent across sampled regions, indicating disparity in the testing of either seroprevalence (this study) or the incident rate (reported by the Saudi MoH).

Table 2
Demographics and chronic diseases in healthcare workers in Riyadh, Saudi Arabia.

	Seronegative N = 275	Seropositive N = 19	P value
Average age	40	37.1	0.3
Male	N (%) 107 (39.4)	N (%) 6 (31.5)	0.49
Diabetes	N (%) 36 (13.2)	N (%) 2 (10.5)	0.73
Hypertension	N (%) 57 (20.9)	N (%) 10 (52.6)	**0.02**
Asthma	N (%) 1 (0.37)	N (%) 1 (5.2)	0.12
Obesity	N (%) 44 (19.3)	N (%) 2 (12.5)	0.29
Depression	N (%) 5 (1.8)	N (%) 1 (5.2)	0.33
Hyperlipidemia	N (%) 58(21.3)	N (%) 5 (26.3)	0.33

Seroprevalence of SARS-CoV-2 in healthcare workers in Riyadh, Saudi Arabia

To estimate the COVID-19 seroprevalence in HCWs in Riyadh city, a total of 400 samples were collected from health practitioners (doctors, nurses, and workers in hospital wards) from two of the main COVID-19 hospitals in Riyadh city; where high number of COVID-19 patients were admitted and treated. Among these HCWs, 7.5% (4.95–10.16 CI 95%) had reactive antibodies to SARS-CoV-2 without reporting any previously confirmed infection (Fig. 3A). Most of HCWs (n = 294) gave access to their clinical and demographic information (Table 2). There was a significant relation between SARS-CoV-2 seropositivity and hypertension in HCWs but no association with any of the remaining co-morbidities such as

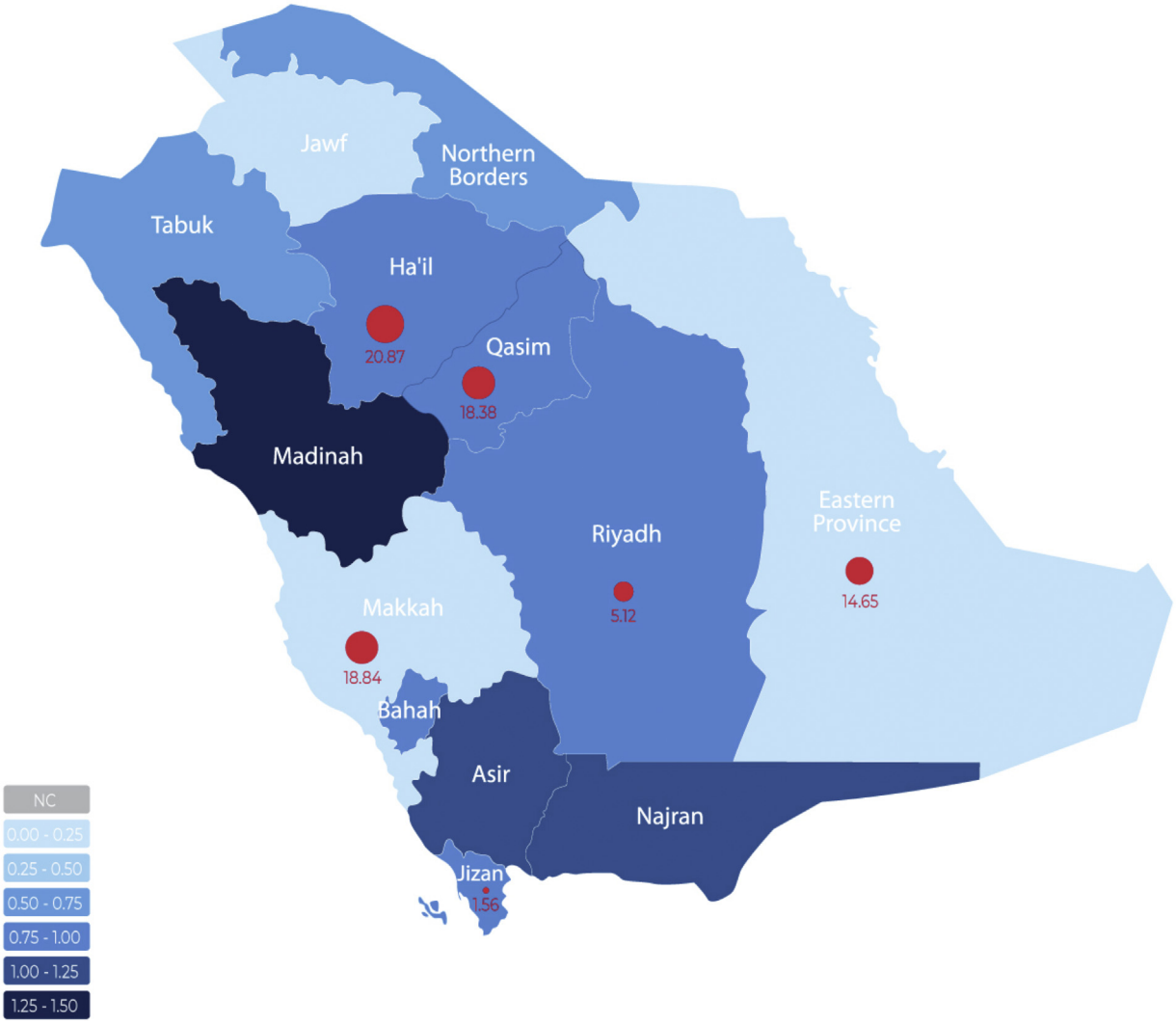

Fig. 2. Estimated seroprevalence rate of COVID-19 and COVID-19 infection rates in all sampled and unsampled Saudi Arabian regions.
Geographical map showing the 13 regions (provinces) of Saudi Arabia. The blue colour intensity represents the COVID-19 incident rates according to the Saudi Ministry of Health [26]; as shown in Table S2. Red circles represent seroprevalence estimates with a circle size relevant to estimates.

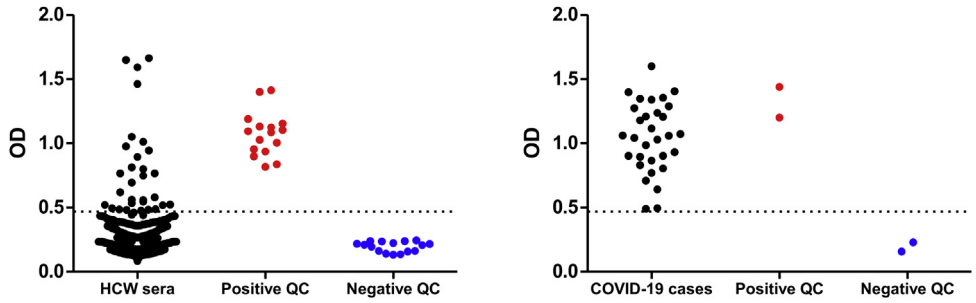

Fig. 3. Anti-SARS-CoV-2 antibodies in samples from COVID-19 patients and HCWs in Riyadh, Saudi Arabia.
In-house ELISA for anti-SARS-CoV-2 spike IgG antibodies in individual serum samples from HCWs (**A**) and acute COVID-19 cases (**B**) with positive and negative controls included as in Fig. 1. The in-house ELISA's cut-off value was calculated as the average of negative controls plus three times the standard deviation.

diabetes and obesity was observed. Data from 31 acute COVID-19 cases, from the same hospitals, are presented along with these HCWs serology data (Fig. 3B).

Discussion

In this study, serum samples were collected from over 11,000 persons in order to estimate the national seroprevalence of COVID-19 in Saudi Arabia; samples were collected from six (out of 13) geographically distributed regions of the country. These regions have a total population of 24.75 million, a majority out of the 34.8 million total Saudi population. A sample pooling strategy was applied to detect anti-SARS-CoV-2 spike IgG antibodies using an in-house ELISA; followed by testing individual samples from some pools to estimate the seroprevalence. The individual sample testing was also performed using different ELISAs that detects anti-SARS-

CoV-2 IgA and IgG. The samples were mainly from healthy blood donors and residual samples from diagnostic laboratories, collected from in- and out-patients (non-COVID-19 patients).

This approach has estimated the overall seroprevalence of COVID-19 in Saudi Arabia to be 11%, ranging from 1.78 to 24.45% in different regions. The seroprevalence among blood donors, non-COVID patients and HCWs are substantial, suggesting high rates of asymptomatic or undiagnosed COVID-19. Given the high testing capacity implemented in KSA, most symptomatic COVID-19 cases would have been captured and reported; and this seroprevalence study would mainly reveal asymptomatic or very mild unreported cases. The severity of COVID-19 cases is associated with earlier antibody induction and higher levels of such antibodies [2]. It has been observed that 40% of asymptomatic cases would become seronegative in two months [7–9]. Therefore, asymptomatic cases may only show low levels of antibodies that would significantly decline in a short time, limiting the utility of seroprevalence to recent asymptomatic cases. Non-COVID patients had a trend of higher seroprevalence than blood donors. This is likely due to their co-morbid conditions as previous studies suggested that comorbidities are risk factors for COVID-19, partially as a result of increased expression of ACE-2, the SARS-CoV-2 host-cell receptor [18,19].

The seroprevalence concomitantly increased with the incident rate of COVID-19. For example, Jazan region was sampled in June and had the lowest seroprevalence, and it has a low incident rate as of December 2020. In unsampled regions, the seroprevalence may vary according to the disease incident. Madinah region had the highest incident rate in KSA (1.27%); this region was not sampled in our study but a recent study has found that the local seroprevalence in Madinah was 19.3% in blood donors [20], falling in a similar range to Makkah region, sampled in the current study, that had 1% incident rate and 18.8% seroprevalence estimate. However, time of serological study is crucial, an early study has reported a seroprevalence of 0% in blood donor samples (n = 956) in Jeddah, collected between January and May 2020 [21]. Nevertheless, the fold increase between seroprevalence and incident rate was not consistent across sampled regions. This is likely due to a bias in the study cohort. It is also possible that mild cases in some regions were not seeking clinical help or testing, especially during the lockdown in KSA (March–June 2020). Although we have no data to support this possibility it warrants further public health investigation. Of note, Saudi Arabia has detected its first COVID-19 case on the 2nd of March 2020; and by 12th of January 2021, the incident rate in the country was around 1% (363,259 confirmed cases in the Saudi population of 34.8 million) as of December 2020 [22].

A number of studies have reported seroprevalence in blood donors, identifying the rate to be 34.2% in Pakistan, 25% in India, 7.7% in Brazil, and 6.5% in the UK; however, only a few studies have reported at a national scale. A total of 14 studies in the UK have identified that the seroprevalence rate is 14%. For residual samples (resembling our non-COVID-19 patient population), studies have identified a rate of 3.97% in the UK and 10.72% in France (SeroTracker.com). SeroTracker.com shows that seroprevalence studies can be very different in terms of serological assays, sample source, geographical coverage, and the population type; in addition, the timing of these studies may only reflect the dynamic transmission of the virus at the time. It may not be practical to compare our data with any other international seroprevalence reports. In Saudi Arabia, there was no previous national report on the general public, blood donors, or residual samples. Studies on HCWs in Saudi Arabia showed that the seroprevalence is 24% in in Madinah city [23] and 2.36% nationally [24]. Our data on HCWs sampled from two of the COVID-19 leading hospitals in the Capital Riyadh showed a rate of 7.5%. Considering age, gender, and co-morbidities in our HCWs, there was a significant indication of an association between seroprevalence in HCWs and prevalence of hypertension. Although this is a small size cohort and requires further studies on a larger sample size, hypertension has been previously reported as a risk factor for COVID-19 [18,25,26].

This study has strengths of sampling largely populated, geographically distributed regions; but it has its limitation of using proxy samples, collect at simple randomization, and it had a pooling strategy that may not reflect precisely the seroprevalence; therefore, it reports seroprevalence estimation. In conclusion, this study estimates the national serological prevalence of COVID-19 in Saudi Arabia to be 11%, with an apparent disparity between regions. This warrants better vaccination programs and vaccination coverage to increase the immuned population.

Author contribution

Sample collection: Areeb Albargawi, Abdullah G. Alharbi, Abdulaziz Alhazmi, Ali Alqarni, Ali Alfarhan, Fayhan Alroqi, Mohammed Bosaeed, Yaseen M. Arabi, Hosam M. Zowawi. *Sample testing:* Hind Albalawi, Mohammed Alenazi, Naif K. Alharbi. *Data analysis and Manuscript Writing:* Naif K. Alharbi, Omar Aldibasi, Suliman Alghnam, Anwar Hashem, Abdullah Algaissi.

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Conflict of interest

The authors declare no conflict of interest.

Ethical approval

The study was approved by the IRB at KAIMRC (Ministry of National Guard Health Affairs) for project number RC20-180. Residual samples from blood banks and clinical laboratories were obtained after an IRB approval. COVID-19 patients and HCWs signed informed consents to give blood samples. The study was also approved by IRB at the Ministry of Health, KSA.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jiph.2021.04.006.

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