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Dramatic rise in seroprevalence rates of SARS-CoV-2 antibodies among healthy blood donors: The evolution of a pandemic

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ABSTRACT

Background: Seroprevalence studies of SARS-CoV-2 antibodies are useful in assessing the epidemiological status in the community, and the degree of spread.
Objective: To study the seroprevalence rates of SARS-CoV-2 antibodies among healthy blood donors in Jordan, at various points of time and as the pandemic evolves in the community.
Methods: In total, 1374 blood donor samples, from three groups, were tested for SARS-CoV-2 total immunoglobulin antibodies. In the first group, samples from 734 individuals (from donations made between January and June 2020) were tested in June. In the second group, 348 individuals were tested in September 2020. The third group of 292 individuals was tested in February 2021. A qualitative assay was used for testing (specificity 99.8%, sensitivity 100%).
Results: The first two groups, from January–June and September 2020, when confirmed Covid-19 cases numbered between several hundred and 3000, showed a seroprevalence rate of 0% (95% CI 0.00–0.51%). The third group (early February 2021), when the number of confirmed cases had reached 100 times that of September 2020, revealed a seroprevalence of 27.4% (95% CI 22.5–32.9%).
Conclusions: A dramatic rise in seroprevalence of SARS-CoV-2 antibodies was seen among healthy blood donors in Jordan, in parallel with widespread intracommunity transmission of the disease. This information is useful for assessing the degree of herd immunity, and provides for better understanding of the pandemic.

Introduction

The coronavirus disease 2019 (COVID-19) pandemic had resulted in more than 147 million cases of confirmed infection, and more than 3 million deaths worldwide as of week 2021-16, 2021 (European Centre for Disease Prevention and Control, 2021).

Population-based seroprevalence studies are extremely important for understanding the evolution of the pandemic, and to estimate infection rates and prevalence. They are also important for calculating absolute risks of infection as well as death rates, and to predict the spread of the virus in communities based on the level of herd immunity following infections and/or vaccination. In addition, they are used for planning and monitoring the impact of implementation and relaxation of epidemic mitigation policies (Busch and Stone, 2021)

The true prevalence of infection is believed to be several times more than the number of PCR-confirmed cases because of the large number of asymptomatic infections and/or mild infections that have gone untested, especially early in the pandemic (Huang et al., 2020; McLaughlin et al., 2020; Busch and Stone, 2021). The ratio of estimated to reported infections can be as much as 12.5 (Bajema et al., 2020).

Numerous population seroprevalence studies have been conducted (Bajema et al., 2020; Lai et al., 2020; Chughtai et al., 2020; McLaughlin et al., 2020; Naranbhai et al., 2020; Sam et al., 2021; Sutton et al., 2020; Menachemi et al., 2020; Silveira et al., 2020; Kar et al., 2021; Vena et al., 2020; Pollán et al., 2020; Bogogiannidou et al., 2020; Poustchi et al., 2020; Ng et al., 2020; Figueiredo-Campos et al., 2020; Stringhini et al., 2020; Havers et al., 2020; Ho et al., 2020; Xu et al., 2020; Qutob et al., 2021; Capai et al., 2020; Sood et al., 2020; Godbout et al., 2020; Rostami et al., 2020) in efforts to estimate the true prevalence of COVID-19 infection.

The largest seroprevalence study was that by Bajema et al., in the USA, which showed that by September 2020 the estimated

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population seroprevalence was less than 10% in the majority of tested communities, although it ranged from 0% up to 23.3% in the hardest-hit areas. This wide variation reflected the levels of transmission in the tested communities and the numbers of PCR-confirmed reported cases (Bajema et al., 2020).

These population-based seroprevalence studies varied in the targeted populations tested and in the recruitment strategies used, which may explain some of the variation. Among the populations tested were healthy blood donors (Amorim Filho et al., 2020; Sughayer et al., 2020; Slot et al., 2020; Nesbitt et al., 2021; Gallian et al., 2020; Olariu et al., 2021; Younas et al., 2020; Banjar et al., 2021; Uyoga et al., 2021; Busch and Stone, 2021; Martinez-Acuña et al., 2020; Saeed et al., 2021; Fiore et al., 2021). Again, the seroprevalence among healthy blood donors and other population groups studied varied according to the community tested and the time of testing, in terms of the pandemic evolution (Lai et al., 2020; Rostami et al., 2020).

The aim of this longitudinal study was to estimate the seroprevalence of Covid-19 among healthy blood donors in Jordan at different points in time, in order to assess the degree of community spread and herd immunity, and to further understand the evolution of the pandemic. To our knowledge, this is the first longitudinal seroprevalence study with blood donors, and the first from Jordan.

Methods

Study population

The study population consisted of three groups of healthy blood donors. The distribution of donors in terms of donation period is detailed in Table 1. Individuals in the first group were randomly chosen from donors attending the blood bank between January and June 2020. The aim was to include donors from January and February 2020, even though the first case of COVID-19 in Jordan was recorded in March of that year. The number chosen from each month was arbitrary. The smallest number in the first group was in June because testing was carried out in the first week of that month. The second and third group were randomly chosen in the first half of September 2020 and late January–early February 2021, respectively. The timing of the second group was chosen to assess the situation when society began to open up again in early September. The third group was chosen to assess the impact of the COVID-19 wave that peaked in January of 2021.

Table 1
Demographics and period of donation of all donors.

Demographics of the 1374 healthy donors, No. (%)	
Male	1177 (85.7%)
Female	197 (14.3%)
Residential area: Amman	1077 (78.4%)
West Amman	706 (65.6%)
East Amman	371 (34.4%)
District outside Amman	297 (21.6%)
North	128 (9.3%)
Center	138 (10.1%)
South	31 (2.3%)
Period	
January 2020	104 (7.6%)
February 2020	89 (6.5%)
March 2020	90 (6.6%)
April 2020	147 (10.7%)
May 2020	229 (16.7%)
June 2020	75 (5.4%)
September 2020	348 (25.3%)
Jan 28–Feb 5, 2021	292 (21.3%)

Samples

Leftover sera and/or plasma collected routinely during the process of blood or apheresis platelet donations were used for the study. The donors were healthy, asymptomatic subjects between the ages of 18 and 63, who underwent routine screening to determine their acceptability for donation, as per standard practice. The sera were tested in three batches at three different points in time. The first batch, consisting of 734 samples representing the period from January to June 2020, was tested in June of that year. The second batch of 348 samples was collected and tested in September 2020, while the third batch of 292 samples was collected during the period January 28–February 5, 2021 and tested in the same period. Thus the total number of donors tested in the study was 1374.

Testing methodology

The samples were tested on Cobas 6000 or Cobas Pro Roche analyzers, using the Elecsys-Anti-SARS-CoV-2 kit (Roche Diagnostics GmbH, Mannheim, Germany), according to the manufacturer's recommendations. This commercially available FDA-approved kit for total immunoglobulins against SARS-Cov-2 is a qualitative assay that uses an electrochemiluminescence immunoassay (ECLIA) for the in vitro detection of total antibodies (IgA, IgM, and IgG) to SARS-CoV-2 in human serum and plasma. The assay uses a recombinant protein representing the nucleocapsid (N) antigen for the determination of antibodies against SARS-CoV-2. The analyzer automatically calculates the cutoff, based on the measurements of two calibrators. The result for a sample is given either as reactive or non-reactive, as well as in the form of a cutoff index (COI; signal sample/cutoff). If the COI is <1.0 then the assay is non-reactive (negative for anti-SARS-CoV-2 antibodies); if COI is ≥ 1.0 then the assay is considered reactive (positive for anti-SARS-CoV-2 antibodies).

The test was validated by the manufacturer using 5272 samples, including blood donors, routine diagnostics, other corona viruses, and common cold panels. The specificity was determined to be 99.8% (95% CI, 99.7–99.9%), while the sensitivity was 100% (95% CI, 88.3–100.0%).

In-house validation using serum samples obtained from previously RT-PCR-confirmed COVID-19-infected patients who had recovered at least 1 month prior to sampling was performed on two occasions: in June 2020 using four samples and in September 2020 using six samples.

Statistical methods

Descriptive statistics were used to analyze the results. The chi-square test was used to compare the seropositive donors' characteristics with the seronegative ones. Student's *t*-test was used to compare the mean ages of the seropositive and seronegative donors. The estimated crude prevalence rate was adjusted to the test's sensitivity and specificity using an online tool (http://www2.univet.hu/users/jreiczig/CI4prevSeSp/calc02/index.php) in line with methods described by Lang and Reiczigel (Lang and Reiczigel, 2014).

Results

The in-house validation of the 10 samples from known confirmed previously infected patients revealed positive results for the presence of total anti-SARS-CoV-2 nucleocapsid antibodies.

The demographics of the donors and the periods of donation are summarized in Table 1. The donors were mostly males (86%) and from the capital city of Amman, Jordan (78%).

Table 2
Comparison of seronegative and seropositive donors in the third group

Category	Seropositive donors 80 of 292	Seronegative donors 212 of 292	*p*-value	Crude prevalence rate for seropositive donors
Number of donors	80 (%)	212 (%)		27.4%
Male	70 (87.5%)	184 (86.8%)	0.9	27.6%
Female	10 (12.5%)	28 (11.8%)		26.3%
Age (yrs) 18–30	47 (58.8%)	116 (54.7%)	0.4	28.8%
31–40	21 (26.3%)	52 (24.5%)		28.8%
41–50	11 (13.7%)	24 (11.3%)		31.4%
51–65	1 (1.2%)	11 (5.2%)		8.3%
Unknown	0	9 (4.2%)		
Blood group: O	33 (41.3%)	88 (41.5%)	0.5	27.3%
A	23 (28.8%)	73 (34.4%)		24.0%
B	15 (18.7%)	37 (17.5%)		28.8%
AB	9 (11.2%)	14 (6.6%)		39.1%
Rhesus blood type: +	70 (87.5%)	194 (91.5%)	0.3	70 (26.5%)
−	10 (12.5%)	18 (8.5%)		10 (35.7%)
Residential location North	7 (8.8%)	33 (15.6%)	0.3	17.5%
Center, including Amman	68 (85.0%)	168 (79.2%)		28.8%
South	5 (6.2%)	11 (5.2%)		31.3%
History of previous COVID-19:				
PCR-confirmed past infection	16 (20%)	0	NA	NA
PCR negative/not performed	47 (58.8%)	180 (84.9%)		
No information available	17 (21.2%)	32 (15.1%)		

The first group was previously reported in a preprint (Sughayer et al., 2020) and, along with the second group, revealed a seroprevalence rate of 0% (95% CI, 0.00–0.51%).

The third group, which represented the most recent period of January 28–February 5, 2021, showed a positive serological test for SARS-CoV-2 in 80 of 292 donors, giving a crude seroprevalence rate of 27.4%. The adjusted estimated seroprevalence rate was 27.3% (95% CI, 22.5–32.9%). The ages of individuals in this group ranged between 18 and 65 years. The mean ages ($\pm$SD) of the seronegative donors and seropositive donors were 31.4 ± 9.75 and 29.7 ± 8.56 years, respectively, with no statistically significant difference between these mean ages (p = 0.13). The demographics and characteristics of the seropositive donors in comparison with the seronegative ones are shown in Table 2. Most of those who tested positive (85%) were in the 18–40 years age group. However, there were no statistically significant differences between the seropositive and seronegative donors in terms of gender, age, blood group, or residence. Males and females were almost equally affected (27.6% vs 26.3%).

One fifth of the seropositive donors were retrospectively found to have been confirmed positive for COVID-19 infection by PCR testing. Forty seven (58.8%) were not known to have had the disease, and so either did not undergo PCR testing or, if they did, produced a negative result. There was no information with regard to previous infections for 17 of the seropositive donors.

Discussion

The importance of serological testing for SARS-CoV-2 antibodies has been previously highlighted (Busch and Stone, 2021; Raoult, 2021). Among the advantages of such testing is an understanding of the evolution of the pandemic in terms of generating a rough estimate of the prevalence of infection. This will help health planners and decision makers to properly enforce or relax mitigation measures. And, most importantly in these times when vaccines are being rolled out, it serves in estimating the risk rates for infection and the degree of herd immunity, and helps in prioritizing vaccine recipients.

In this study the seroprevalence rates in healthy blood donors were measured at three points in time. The results are striking in that they show a dramatic change from 0% early and in the middle of the pandemic, up to 27.4% in February 2021. These findings would seem reasonable if we consider the cumulative number of confirmed cases around these times in Jordan. Figure 1 shows the cumulative daily cases of COVID-19 in Jordan. In the first period up to June 2020 there were only several hundred confirmed cases, which increased gradually to around 3000 cases in early September (WHO, 2020). However, towards the end of September and beyond the cumulative number of cases started a steep rise such that, by February 5, 2021, the number had increased 100-fold since September 2020. It is worth mentioning that a strict lockdown was in effect until early June, which was gradually relaxed over the following 3 months, with full opening of all sectors, including schools and international travel, in September 2020. It is clear that the first wave of the COVID-19 pandemic in Jordan actually started in late September when the community was fully open and the intracommunity spread became evident. Before that, the several hundred cases were limited to transmission within known specific hot foci. This explains the extremely low seroprevalence initially found in June and September of 2020, as the infection transmission was under strict control with quarantine routinely imposed on all contacts of index cases.

The crude seroprevalence rate found in early February 2021 of 27.4%, if generalized to the entire Jordan population, would mean that the number of cases was roughly 2.7 million in a population of 10 million. If true, this would mean that there were eight cases for every confirmed case. This high ratio of estimated to reported cases

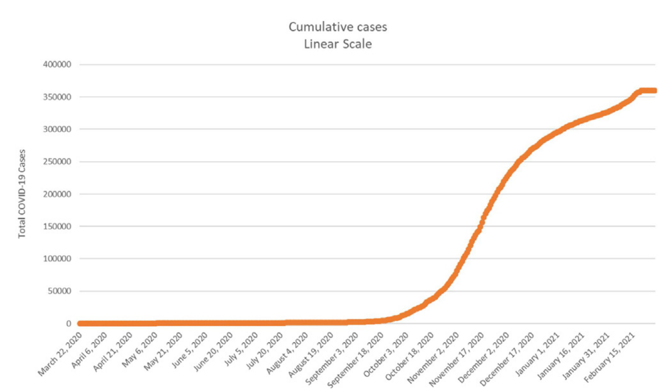

Figure 1. Cumulative number of confirmed COVID-19 cases in Jordan.

is similar to some of the highest ratios reported by Bajema et al. in the USA (Bajema et al., 2020). Looking at it from a different perspective, we can also see that in our rather small sample, one fifth of the donors had been previously confirmed positive by the PCR test, so we may assume a ratio of estimated to reported cases of five, as a lowest estimate.

Most of our seropositive donors (85%) were young — below 40 years of age. Other studies have shown inconsistent findings, with either similar age group distributions to our cohort or the opposite situation, with higher rates in older individuals (Bajema et al., 2020; Martinez-Acuña et al., 2020; Vena et al., 2020; Olariu et al., 2021). Nevertheless, this finding regarding age helps in setting vaccination priorities for those older than 40 years.

Our findings were also in line with previous studies, which showed that early in the pandemic the seroprevalence of COVID-19 in blood donors or other nontargeted populations was very low, ranging from 0% to 2.0% (Erikstrup et al., 2021; Godbout et al., 2020; Qutob et al., 2021; Xu et al., 2020; Ho et al., 2020; Banjar et al., 2021; Nesbitt et al., 2021; Slot et al., 2020; Fiore et al., 2021; Saeed et al., 2021). However, other studies, especially those from hard-hit communities early in the pandemic, showed relatively high rates of up to 23% (Rosenberg et al., 2020; Percivalle et al., 2020).

The high seroprevalence rate found in our study for early February 2021 was similar to that for communities that were severely affected by the pandemic, such as those of New York City and Chelsea, Massachusetts (Bajema et al., 2020; Naranbhai et al., 2020).

With regard to blood group, in terms of a decreased risk for COVID-19 infection in those of blood group O (Gallian et al., 2020), our limited data did not reveal such an association.

To our knowledge, our study was the first longitudinal study of COVID-19 seroprevalence in healthy blood donors. The results illustrate the evolution of the pandemic, and the dynamics and hidden magnitude of the problem.

There were some limitations in our study, including the small number of blood donors in the third group. In addition, although the age groups represented in our study constituted around 60% of the Jordanian population, with the rest being mostly children under the age of 18, the blood donors were mostly males and of younger age groups. Thus, it may be difficult to draw generalizations, as there may have been selection bias and nonrepresentation of the entire population. Further studies are recommended to include children and the elderly.

In conclusion, a dramatic rise in seroprevalence of SARS-CoV-2 from almost 0% to 27.4% in healthy blood donors was seen as the Jordanian community entered into the peak of the first wave of COVID-19. An estimate of the true prevalence was achieved through this longitudinal serological study, leading to much better insight and understanding of the evolution of the pandemic in Jordan.

Several gaps are present in this study. These include the limitations previously mentioned, especially the small number of the donors, the bias with regard to age and gender, and the consequent lack of full representation of the entire community. Nevertheless, this study represents an important addition to our means of controlling and fighting COVID-19.

Conflicts of interest

The authors declare no conflicts of interest.

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Ethical approval

The institutional review board (IRB) at King Hussein Cancer Center approved the study.

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