Seroprevalence of antibodies to SARS-CoV-2 among blood donors in the early month of the pandemic in Saudi Arabia

Ayman Banjar[1], Jaffar A. Al-Tawfiq[2,3,4*], Amaal Alruwaily[5], Haleema Alserehi[5], Ada Al-Qunaibet[5], Rehab Alaswad[5], Hind Almutlaq[5], Abdullah Almudaiheem[5], Abdullah T. Khojah[6], Faisal Alsaif[7], Shaza Karim Almolad[5], Saeed Alqahtani[8], Abdullah AlJurayyan[9], Abdullah Alotaibi[10], Safar Almalki[11], Yousef Abuhaimed[12], Abdullah Alkhashan[8], Amal Alfaifi[1], Khaled Alabdulkareem[1,6], Hani Jokhdar[1], Abdullah Assiri[1], Sami Almudarra[1,5]*

Affiliations:

[1]Deputy Ministry for Public Health, Ministry of Health, Riyadh, Saudi Arabia; [2]Infectious Disease Unit, Specialty Internal Medicine, Johns Hopkins Aramco Healthcare, Dhahran, Saudi Arabia, [3]Infectious Disease Division, Department of Medicine, Indiana University School of Medicine, Indianapolis, IN (USA), [4]Infectious Disease Division, Department of Medicine, Johns Hopkins University, Baltimore, MD; [5]Saudi Center for Disease Prevention and Control, Riyadh, Saudi Arabia; [6]Faculty of Medicine, Al Imam Muhammad ibn Saud Islamic University, Riyadh, Saudi Arabia; [7]Faculty of Medicine, King Saud University, Riyadh, Saudi Arabia; [8]Deputy Ministry for Laboratories and Blood Banks, Ministry of Health, Riyadh, Saudi Arabia; [9]King Fahad Medical City, Ministry of Health, Riyadh, Saudi Arabia; [10]Dammam Regional Laboratory, Ministry of Health, Dammam, Saudi Arabia; [11]Jeddah Regional Laboratory, Ministry of Health, Jeddah, Saudi Arabia; [12]King Saud bin Abdulaziz University for Health Science, Riyadh, Saudi Arabia

* Corresponding Author:

Sami Almudarra, MD, PhD; Saudi Center for Disease Prevention and Control, Riyadh, Saudi Arabia

E-mail address: salmudarra@moh.gov.sa

OR

Professor Jaffar A. Al-Tawfiq

P.O. Box 76; Room A-428-2, Building 61, Dhahran Health Center, Johns Hopkins Aramco Healthcare, Dhahran 31311, Saudi Arabia.

E-mail address: jaltawfi@yahoo.com

Highlights (mandatory)

- Among blood donors in May 2020, te overall seroprevalence of anti-SARS-CoV-2 antibodies was 1.4%
- Non-citizens had higher prevalence compared to citizens (odds ratio 13.6, p = 0.001).
- There was geographic variation in seroprevalence of anti-SARS-CoV-2 antibodies within Saudi Arabia
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Keywords: serology; COVID-19; SARS-CoV-2; Blood donors; IgG antibody; Seroprevalence

ABSTRACT

BACKGROUND: Serologic testing provides better understanding of SARS-CoV-2 prevalence and its transmission. This study is the first investigation of the prevalence of antibodies to SARS-CoV-2 among blood donors in Saudi Arabia.

OBJECTIVE: To estimate the seroprevalence of anti-SARS-CoV-2 antibodies among blood donors in Saudi Arabia during the early phase of the current pandemic.

METHODS: Serology results and epidemiological data were analyzed for 837 adult blood donors, with no confirmed SARS-CoV-2 infection, in Saudi Arabia from 20 to 25 May 2020. Seroprevalence was determined using electrochemical immunoassay for detecting anti-SARS-CoV-2 antibodies.

RESULTS: The overall seroprevalence of anti-SARS-CoV-2 antibodies was 1.4% (12/837). Non-citizens had higher prevalence compared to citizens (odds ratio 13.6, p = 0.001). Having a secondary education as the highest level of education was also significantly associated with high seroprevalence compared to higher education (odds ratio 6.8, p = 0.005). The data showed that the highest seroprevalence was in Makkah (8.1%), Madinah (4.1% with an OR of 0.48 (95% CI: 0.12-1.94), Jeddah (2.3%; OR 0.27; 95%CI: 0.31-2.25), and Qassim (2.9 %, OR: 0.34; 95%CI: 0.04-2.89) and these were not statistically different from the seroprevalence in Makkah region.

CONCLUSIONS: At the early stage of the pandemic in Saudi Arabia, the seroprevalence of antibodies to SARS-CoV-2 among blood donors was low but was higher among non-citizens These findings showed that non-citizens and the less educated may be less attentive to preventive measures. Monitoring seroprevalence trend over time require repeated sampling.

INTRODUCTION

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is a novel coronavirus causing CoronaVirus Disease 19 (COVID-19) infection at a pandemic scale and with rapid spread and was first detected in Wuhan city, China in December 2019 (AlJishi and Al-Tawfiq, 2020; Drosten et

al., 2015). COVID-19 has a wide clinical spectrum from an asymptomatic or mild infection in most cases to a severe acute respiratory syndrome (SARS) (Al-Tawfiq, 2020).

The first confirmed COVID-19 case in Saudi Arabia was reported on March 2nd, 2020 (Al-Tawfiq and Memish, 2020a; AlJishi et al., 2021); and by May 20th, 2020, the reported cases reached 62545 nationally and more than 4.8 million cases globally (World Health Organization(WHO), 2020). The main diagnostic tests for the detection of SARS-CoV-2 infection relies on molecular diagnostic tests, namely reverse transcription polymerase chain reaction (RT-PCR) (Al-Tawfiq and Memish, 2020b; Gronvall et al., 2020). The use of serologic tests is important to understand the extent and prevalence of COVID-19 infections (Al-Tawfiq and Memish, 2020b). In addition, such tests are needed to determine the proportion of the population that has an immune response to SARS-CoV-2.

The clinical presentation and epidemiology of COVID-19 infection in Saudi Arabia parallel those from around the globe (Al-Omari et al., 2020; Al Mutair et al., 2020). To understand the extent of the disease in Saudi Arabia, the Ministry of Health (MoH) and the Saudi Center for Disease Prevention and Control (SCDC) conducted a nationwide serologic testing early May 2020 to estimate the prevalence of antibodies to SARS-CoV-2 in multiple settings and population including social care homes, rehabilitation centers, healthcare workers (Al Mutair et al., 2020), and blood donors. The advantage of utilizing blood banks for seroprevalence studies is that they usually have frequent number of donors that could facilitate serial cross-sectional studies at multiple times.

This study is the first investigation of the seroprevalence of antibodies to SARS-CoV-2 among blood donors in Saudi Arabia. The objectives of the study were to help determine the

seroprevalence and to support the understanding of the epidemiological characteristics including factors associated with infection.

METHODS

This is a cross-sectional study of SARS-CoV-2 antibody in blood donors. All blood donors irrespective of their sociodemographic characteristics who presented for blood donation at one of 24 main blood banks in Saudi Arabia were included in the study. Blood donors with a contraindication for blood donation, those with a past or current confirmed SARS-CoV-2 infection, and those who declined to give informed consent to participate in the study were excluded.

The participants were included in the study from 20 to 25 May 2020. The current study was conducted at the time when the Kingdom of Saudi Arabia had 62,000-80,000 cases and just before the peak of cases in the country (Alserehi et al., 2020). All participants signed informed consents, and completed the study questionnaire.

Study Questionnaire

Each participant was instructed to complete a written questionnaire which covers demographic (age; sex; citizenship status; education level), clinical data (current symptom and date of onset; health conditions; and smoking behavior), and exposure characteristics (contacted a confirmed or suspected SARS-CoV-2 case, attended a gathering, and/or visited a healthcare setting during the last 14 days; and SARS-CoV-2 RT-PCR testing). Location information of the blood bank site and the province were also collected.

Serum Sample Collection

Before beginning blood donation, 4 ml of blood samples were collected in a serum collection tube from each participant by a trained blood bank staff. All collected serum samples were transported and stored for antibody testing at one of the designated ministry of health (MoH) regional laboratories in the cities of Jeddah or Dammam per the study protocol and manufacturer's instructions.

Serological Testing

To detect anti-SARS-CoV-2 antibodies, an electrochemiluminescent immunoassay (ECLIA) was performed using the Elecsys® Anti-SARS-CoV-2 test from Roche Diagnostics International Ltd. (Rotkreuz, Switzerland) for the qualitative detection of antibodies (including IgG) to SARS-CoV-2. The test is based on in-solution double-antigen sandwich format that can detect antibodies using a recombinant protein representing the nucleocapsid (N) protein of SARS-CoV-2 (Lau et al., 2020; Migchelsen and Duggan, 2020; Muench et al., 2020). The test has 99.8% sensitivity (14 days after a PCR-confirmed infection) and 99.5% specificity (with limited cross-reactivity of 4/792 (0.5%) to the 4 human coronaviruses causing common cold) (Muench et al., 2020). The result of a sample is given either as reactive (i.e., positive for anti-SARS-CoV-2 antibodies if the result's cut-off index is $\geq$1.0) or non-reactive (i.e., negative for anti-SARS-CoV-2 antibodies if the result's cut-off index is <1.0)(Migchelsen and Duggan, 2020).

Statistical Analysis:

Descriptive analysis was used to summarize the data using range and mean ± standard deviation (SD), as appropriate for continuous variables and proportion (%) for categorical variables. Inferential analysis was performed using t-test and chi-squared test as appropriate to compare participants' demographic, clinical, and exposure characteristics between seropositive and

seronegative participants. Logistic regression was then performed to establish the correlates of seroprevalence using odds ratio (OR) for the statistically significant characteristics. STATA® (Stata/IC software version 15.1) from StataCorp LLC. (College Station, Texas, USA) was used for performing statistical analysis. A two tailed p-value (p) <0.05 was considered statistically significant and 95% confidence interval (CI) was used, as appropriate, to report the estimates.

Ethical Considerations

This study was approved by the MoH's Central Institutional Review Board (log number: 20-107M) according to the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH)'s Good Clinical Practice (GCP) guidelines. The study national registration number at the National Committee of BioEthics, King Abdulaziz City for Science and Technology, Saudi Arabia is H-01-R-009.

RESULTS:

In total, 837 participating blood donors, aged 17 to 70 years, were included in the study. Anti-SARS-CoV-2 antibodies were detected in 12 (1.4%) of all participants (Table 1). Males constituted 96% of the study participants and all seropositive participants. National citizens formed 72% of the study participants but only 17% of the seropositive participants.

There was no significant difference for mean age or prevalence in the different age group of seropositive and seronegative participants (table 1). Other characteristics that had no significant difference for seroprevalence included gender, having current symptom, having a health condition, being a smoker, previous exposure to SARS-CoV-2 and/or a high-risk event in the last 14 days, or had SARS-CoV-2 RT-PCR testing. By contrast, citizenship status, education level, and directorate of health affairs (a proxy measure for the home address of the blood donor) had significant

difference for the prevalence of anti-SARS-CoV-2 antibodies between the seropositive and seronegative participants with p-value <0.001, 0.005, and 0.002, respectively.

In logistic regression analysis, non-citizen participants had higher odds ratio of being seropositive than citizens (OR 13.6, 95% CI 2.96-62.75, p = 0.001) (table 2). Education level was also significantly associated with seroprevalence with participants reporting secondary education as the highest level of educational had higher odds than those reporting higher education (OR 6.8, 95% CI 1.79-25.95, p = 0.005). The data showed that the highest seroprevalence was in Makkah (8.1%), Madinah (4.1% with an OR of 0.48 (95% CI: 0.12-1.94), Jeddah (2.3%; OR 0.27; 95%CI: 0.31-2.25), and Qassim (2.9%, OR: 0.34; 95%CI: 0.04-2.89) and these were not statistically different from the seroprevalence in Makkah Region (table 2).

DISCUSSION

This study was conducted as part of Saudi Arabia nationwide serosurvey estimating the prevalence of antibodies to SARS-CoV-2 in multiple population groups that started around May 20th, 2020. The study investigated the seroprevalence among blood donors with no previously confirmed infection and explored their epidemiological characteristics.

The overall prevalence of antibodies to SARS-CoV-2 among the participants was 1.4% with substantial variation in the different cities. The low prevalence of antibodies might not indicate a true low prevalence of past infection. In a review article (Tirupathi et al., 2020b), it was noted that antibody response can decrease in one to three months after acute infection (Long et al., 2020; Robbiani et al., 2020) and that the level of antibodies, persistence and duration of antibodies may differ among different patients (Lee et al., 2020; To et al., 2020; Xu et al., 2020). This overall seroprevalence rate is lower than the rate of 2.36% among healthcare workers in Saudi Arabia

(Alserehi et al., 2020). For non-citizens, the probability of testing positive for anti-SARS-CoV-2 antibodies was 13.6 times higher than for citizens. The difference in seroprevalence among citizens and non-citizens is likely related to the fact that there was a difference in the initial number of cases in Saudi Arabia. The initial cases were initially reported more among non-citizens due to sociodemographic characteristics. A similar disparity in the positivity rate of acute COVID-19 infection was noted among different race and ethnicities (Tirupathi et al., 2020a). Racial and ethnic minority families may live in congested and overcrowded households and this may lead to increased likelihood of contracting SARS-CoV-2 (Tirupathi et al., 2020a). The difference in serology among different regions and among non-citizens compared to citizens may shed light on priorities for vaccine. However, there are multiple factors that are being considered for prioritizing the vaccine based on risk of exposure and risk of disease and its associated morbidity and mortality. Also, the probability was 6.8 times higher for participants having secondary education than for those having higher education. The study found no significant association between the location of the directorate of health affairs and seroprevalence and no significant difference in seropositivity for the other characteristics including age group and sex.

The seroprevalence in this study, albeit conducted among blood donors who had no confirmed SARS-CoV-2 infection, was consistent with most findings from other studies of the prevalence of anti-SARS-CoV-2 antibodies among blood or plasma donors, or of stored samples, from January to June 2020. Notwithstanding the differences in study methods and SARS-CoV-2 transmission patterns between different areas, most of these studies found low levels of seroprevalence of 0% to 23% (Erikstrup et al., 2020; Gallian et al., 2020; Grzelak et al., 2020; Luiz Amorim Filho1, Célia Landmann Szwarcwald, Sheila de Oliveira Garcia Mateos, Antonio Carlos Monteiro Ponce de Leon, Roberto de Andrade Medronho, Valdiléa Gonçalves Veloso, Josiane Iole França Lopes,

Luis Cristovão de Moraes Sobrino Porto, Alexandre Ch, 2020; Ng et al., 2020; Percivalle et al., 2020; Sughayer et al., 2020; Thompson et al., 2020; Valenti et al., 2020).

For example, 0% seroprevalence was reported in Amman city, Jordan (Sughayer et al., 2020); 0.1% in the San Francisco Bay Area, California, USA (Ng et al., 2020); 0.9% (IgG) in 3 federal states (North Rhine-Westphalia, Hesse, and Lower Saxony) in Germany (Fischer et al., 2020); 1.9% in Denmark (Erikstrup et al., 2020); 2.7% in blood donors in the Netherlands (Slot et al., 2020b); 2.7% in 4 departmental areas in France (Gallian et al., 2020); 3.0% in in two blood banks in Clermont and Noyon cities, Oise, France (Fontanet et al., 2020); 3.2% in Scotland (Thompson et al., 2020); 4.0% in the State of Rio de Janeiro, Brazil (Luiz Amorim Filho1, Célia Landmann Szwarcwald, Sheila de Oliveira Garcia Mateos, Antonio Carlos Monteiro Ponce de Leon, Roberto de Andrade Medronho, Valdiléa Gonçalves Veloso, Josiane Iole França Lopes, Luis Cristovão de Moraes Sobrino Porto, Alexandre Ch, 2020); 7.1% in Milan metropolitan area, Lombardy, Italy (Percivalle et al., 2020; Valenti et al., 2020); and 8.5% (overall weighted adjusted) in England (Erikstrup et al., 2020; Gallian et al., 2020; Grzelak et al., 2020; Luiz Amorim Filho1, Célia Landmann Szwarcwald, Sheila de Oliveira Garcia Mateos, Antonio Carlos Monteiro Ponce de Leon, Roberto de Andrade Medronho, Valdiléa Gonçalves Veloso, Josiane Iole França Lopes, Luis Cristovão de Moraes Sobrino Porto, Alexandre Ch, 2020; Ng et al., 2020; Percivalle et al., 2020; Sughayer et al., 2020; Thompson et al., 2020; Valenti et al., 2020).

One study reported 23.0% seroprevalence in the Lodi Red Zone (in Lombardy region) in Italy which was highly affected by the pandemic and under complete lockdown (Percivalle et al., 2020). In this study, Makkah's directorate of health affairs, the health authority for the city of Makkah and its surrounding areas, had the highest proportion of seropositivity with 8.1% prevalence compared to the other participating directorates. The city of Makkah itself was also highly affected

and under complete lockdown and had curfew for a longer time than the other cities in the country (Algaissi et al., 2020; Alserehi et al., 2020; Obeid et al., 2020). In addition, Madinah had 14.6% of the cases and had 4.1% positivity rate. On the other hand, the Eastern Province had about 19% of the cases and Riyadh had 20.2% of the cases with no positivity among blood donors at that time. The current study found a prevalence of 4.1% in Madinah. A recent study showed a prevalence of 19.3% and that study was Mid-May to Mid-July 2020. Thus, the difference could be related to the difference in the timing of these two studies (Mahallawi and Al-Zalabani, 2020). Thus, other factors may had played in the contribution to the differences in the serology among blood donors.

In the current study, we found that seroprevalence among secondary education was higher than those with higher education (OR 6.8, 95% CI 1.79-25.95, p = 0.005). Similarly, in a study from the State of Rio de Janeiro in Brazil, there was a significant association between lower educational level among blood donors and higher prevalence of antibodies to SARS-CoV-2 (Luiz Amorim Filho1, Célia Landmann Szwarcwald, Sheila de Oliveira Garcia Mateos, Antonio Carlos Monteiro Ponce de Leon, Roberto de Andrade Medronho, Valdiléa Gonçalves Veloso, Josiane Iole França Lopes, Luis Cristovão de Moraes Sobrino Porto, Alexandre Ch, 2020).

We found no significant difference for mean age or prevalence in the different age group of seropositive and seronegative participants. In a study from the Netherlands, a significant positivity rate was found among younger donors aged 18-30 years with a prevalence of 4.2% compared to about 2.7% in other age groups(Slot et al., 2020a). In addition, a significant association was found across geographical areas (Erikstrup et al., 2020). Samples collected at later periods during study conduction were significantly associated with an increase in seroprevalence in the studies from Brazil and Milan metropolitan area in Italy (Luiz Amorim Filho1, Célia Landmann Szwarcwald, Sheila de Oliveira Garcia Mateos, Antonio Carlos Monteiro Ponce de Leon, Roberto de Andrade

Medronho, Valdiléa Gonçalves Veloso, Josiane Iole França Lopes, Luis Cristovão de Moraes Sobrino Porto, Alexandre Ch, 2020; Valenti et al., 2020).

Therefore, to monitor SARS-CoV-2 spread and seroprevalence trend over time, nationwide repeated cross-sectional study is needed to better determine how is the prevalence changing in different areas of the country, especially if reported cases do not reflect the true scale of the pandemic, and to ascertain the cumulative population immunity. Such epidemiologic study was done in different countries. For example, the seroprevalence was 4.8% in the first week, 8.5% in the second week, 10.9% in the third week, 6.6% in the fourth week, and 10.8% in the fifth week in Switzerland (Fontanet et al., 2020). However, the interpretation of serology tests so far is problematic especially with the fact that antibodies may not last for a long time. One study showed that antibody response remains stable for two months (Fontanet et al., 2020).

In conclusion, in the early stage of the pandemic in Saudi Arabia, the seroprevalence of antibodies to SARS-CoV-2 among blood donors was low but was higher among non-citizens and in high prevalence areas. Monitoring seroprevalence trend over time require repeated sampling and testing to characterize the prevalence of COVID-19. With the introduction of vaccination, such serologic testing would need to be looked at carefully.

Authors Agreement

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Conflict of Interest

The authors declare no conflict of interest.

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1. Characteristics of participating blood donors and seroprevalence of anti-SARS-CoV-2 antibodies according to characteristics, Saudi Arabia, 20-25 May 2020.

Characteristic	Number of Participants	Seronegative participants	Seropositive participants	Prevalence (%)	*p*[1]
Anti-SARS-CoV-2 test result	837	825	12	1.4	-
Age[2] range (years)	17-70	17-70	25-50	-	-
Age[2], mean (SD) (years)	33.3 (8.3)	33.2 (8.3)	37.4 (8.4)	-	0.081
Age[2] group (years)	-	-	-	-	0.723
17-19	23	23	0	0.0	-
20-29	256	253	3	1.2	-
30-39	381	377	4	1.0	-
40-49	135	131	4	3.0	-
50-59	34	33	1	2.9	-
60-69	2	2	0	0.0	-
70+	1	1	0	0.0	-
Sex[3]	-	-	-	-	0.484
Male	796	784	12	1.5	-
Female	32	32	0	0.0	-
Citizenship status[4]	-	-	-	-	<0.001
Citizen	605	603	2	0.3	-

Non-citizen	231	221	10	4.3	-
Education level[5]	-	-	-	-	0.005
Higher education	540	537	3	0.6	-
Secondary education	218	210	8	3.7	-
Lower education	75	74	1	1.3	-
Directorate of health affairs[6]	-	-	-	-	0.002
Aseer	51	51	0	0.0	-
Baha	31	31	0	0.0	-
Bisha	31	31	0	0.0	-
Eastern Region	53	53	0	0.0	-
Hafr Al Batin	31	31	0	0.0	-
Hail	2	2	0	0.0	-
Hasa	43	43	0	0.0	-
Jazan	42	42	0	0.0	-
Jeddah	43	42	1	2.3	-
Madinah	73	70	3	4.1	-
Makkah	86	79	7	8.1	-
Najran	43	43	0	0.0	-
Northern Borders	23	23	0	0.0	-
Qassim	34	33	1	2.9	-

Qunfudhah	23	23	0	0.0	-
Qurayyat	22	22	0	0.0	-
Riyadh	116	116	0	0.0	-
Tabouk	29	29	0	0.0	-
Taif	61	61	0	0.0	-
Current symptom[7]	-	-	-	-	0.574
No	808	796	12	1.5	-
Yes	21	21	0	0.0	-
Health condition[8]	-	-	-	-	0.673
No	819	807	12	1.5	-
Yes	12	12	0	0.0	-
Smoker[9]	-	-	-	-	0.067
No	547	536	11	2.0	-
Yes	269	268	1	0.4	-
Exposure history (contacted confirmed or suspected COVID-19 case, attended gathering, and/or visited healthcare setting during last 14 days)	-	-	-	-	0.943
No	691	681	10	1.5	-

Yes	**146**	**144**	**2**	**1.4**	**-**
SARS-CoV-2 RT-PCR testing[10]	-	-	-	-	0.231
No	747	735	12	1.6	-
Yes	88	88	0	0.0	-

Abbreviations: COVID-19 = coronavirus disease 2019; ND = no data; *p* = *p*-value; RT-PCR = reverse transcription polymerase chain reaction; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2; SD = standard deviation.

[1] 5% significance level. Difference in mean age of seropositive and seronegative participants was tested using *t*-test. Differences in proportion of all other characteristics were tested using chi-squared test especially Pearson's chi-squared test.

[2] Age was unknown for 5 seronegative participants who were excluded from the analysis.

[3] Sex was unknown for 9 seronegative participants who were excluded from the analysis.

[4] Citizenship status was unknown for 1 seronegative participant who was excluded from the analysis.

[5] Education level was unknown for 4 seronegative participants who were excluded from the analysis.

[6] Serology samples were missing for Jouf's directorate of health affairs in which they were excluded from the study.

[7] Current symptoms were unknown for 8 seronegative participants who were excluded from the analysis. The list of symptoms in the study questionnaire included cough, fever (>38.0˚ Celsius) or feeling feverish, headache, joint pain, nasal drip, nausea and/or vomiting, and sore throat. Bone pain was reported by 1 participant as other symptoms.

[8] Health conditions were unknown for 6 seronegative participants who were excluded from the analysis. The list of health conditions in the study questionnaire included cardiac disease, chronic lung disease, diabetes, high blood pressure, and obesity. Asthma was reported by 1 participant as other health conditions.

[9] Smoking behavior was unknown for 21 seronegative participants who were excluded from the analysis.

[10] Blood donors with confirmed SARS-CoV-2 infection were excluded from participation in the study. History of SARS-CoV-2 RT-PCR testing was unknown for 2 seronegative participants who were excluded from the analysis.

Table 2. Association of participating blood donors' characteristics with the seroprevalence of anti-SARS-CoV-2 antibodies, Saudi Arabia, 20-25 May 2020.

Characteristic	Category	Number of Participants	Prevalence (%)	OR	95% CI	*p*[1]
Citizenship status	**Citizen**	605	0.3	1.00	Ref.	-
	Non-citizen	231	4.3	13.64	2.96-62.75	0.001
Education level	**Higher education**	540	0.3	1.00	Ref.	-
	Secondary education	218	4.3	6.81	1.79-25.95	0.005
	Lower education	75	1.3	2.41	0.24-23.55	0.447
Directorate of health affairs	**Makkah**	86	8.1	1.00	Ref.	-
	Madinah	73	4.1	0.48	0.12-1.94	0.306
	Jeddah	43	2.3	0.27	0.31-2.25	0.226
	Qassim	34	2.9	0.34	0.04-2.89	0.324

Abbreviations: CI = confidence interval; OR = odds ratio; *p* = *p*-value; Ref. = reference; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.

[1] 5% significance level. Differences are relative to the variable listed first as a reference in each characteristic.