Infectious Diseases Diagnosis & Treatment
AL-Hmoud N, et al. Infect Dis Diag Treat 7: 230.
www.doi.org/ 10.29011/2577- 1515.100230
www.gavinpublishers.com

OPEN ACCESS

GAVIN PUBLISHERS

Research Article

Seroprevalence of SARS-CoV-2 Specific IgG Antibodies among a Scientific Community in Jordan during the Second Pandemic Wave

Nisreen AL-Hmoud[1], Mumen Alrwashdeh[1], Amany Abdien[1], Ghadeer Alzghoul[1], Bassam Hayek[1,2]

[1]The Bio-Safety & Bio-Security Centre, The Royal scientific Society, Amman 11941, Jordan

[2]Al Hayek for Environmental Consultancy, Amman 11183, Jordan

***Corresponding author:** Nisreen D. AL-Hmoud, The Bio-Safety & Bio-Security Centre, Royal scientific Society, Amman 11941, Jordan.

Citation: AL-Hmoud N, Alrwashdeh M, Abdien A, Alzghoul G, Hayek B (2023) Seroprevalence of SARS-CoV-2 Specific IgG Antibodies among a Scientific Community in Jordan during the Second Pandemic Wave. Infect Dis Diag Treat 7: 230. DOI: 10.29011/2577-1515.100230

Received Date: 30 August 2023; **Accepted Date:** 11 September 2023; **Published Date:** 14 September 2023

Abstract

Coronavirus Disease "COVID-19" is a new human pandemic disease has spreaded worldwide since late of 2019. SARS-CoV-2, the causative agent of COVID-19, is an emerging human coronavirus that has infected more than 572 million people around the world and caused more than 6.3 million deaths by July 2022. Seroprevalence study is an important tool to assess the level of social immunity within a target population. A total of 937 blood samples were collected from the employees of the Royal Scientific Society of Jordan in the period between January 10th and February 4th, 2021. Serum samples were harvested from the blood samples and preserved in appropriate conditions. Enzyme Linked-Immunosorbent Assay "ELISA" technique was used to detect the COVID-19 IgG antibodies in the serum samples. Demographical data and medical history data were collected from all participants to subsequently interpret the results. Out of 937, 243 (25.93%) were tested seropositive for the COVID-19 specific IgG antibodies. Surprisingly, 121 out of those tested seropositive (49.79%) were not previously tested positive for COVID-19 causative agent via PCR test. On the other hand, 26 individuals who tested positive via PCR test for COVID-19, were tested seronegative for COVID-19 specific IgG antibodies (2.77%). The results of this study revealed the significant number of undiscovered COVID-19 cases within a specific community. The study also showed the importance of conducting seroprevalence studies on national-scale in Jordan; as such studies will give a clear assessment on the Jordanian population immunity against COVID-19. In addition to help in sustaining work in many sectors in the country.

Keywords: Antibodies; COVID-19; ELISA; Jordan; Seroprevalence

Introduction

In December 2019, a novel human coronavirus was detected in people who had visited a seafood market in Wuhan, China [1,2]. The virus belongs to the Beta coronaviruses genus, which originates in animals and infects people, and it is extremely similar to the SARS-CoV virus that caused an outbreak in 2002-2003 [3,4]. SARS-CoV-2, as currently known, causes coronavirus disease (COVID-19) and was declared as a pan-demic in March 2020 [5]. More than 572 million COVID-19 cases worldwide have been reported to WHO to date (July 2022), with over 6.3 million deaths [6]. Jordan reported the first verified COVID-19 case on 2 March 2020, to a man at middle age who recently came from Italy [7]. After one year of reporting the first COVID-19 case in Jordan, the country was rated one of the highest countries for reporting

daily COVID-19 cases in comparison to the number of populations in March 2021 [8]. Heretofore, Jordan has reported more than 1.7 million cases and more than 14 thousand deaths [9]. Since late 2020, when Pfizer Company announced about its mRNA vaccine [10], several vaccine candidates have successfully passed clinical phase trials. WHO issued seven COVID-19 vaccines that were approved for Emergency Use Listing until November 2021 [11]. Pfizer/BioNTech, Sputnik V, Oxford/AstraZeneca, and Sinopharm are the only COVID-19 vaccines that Jordan has approved for citizens [12,13]. On January 13th 2021, Jordan launched a national immunization campaign against COVID-19 targeting adults over the age of 60 years and front-line healthcare workers. In the early stages of the campaign, many people were unwilling to obtain the vaccine shots. By December 2021, more than 3.7 million persons received two doses of COVID-19 vaccination and more than 4 million Jordanians had received at least the first dose [9], out of 10.8 million of the total population of Jordan (According to the Department of Statistics-Jordan 2020). It is still unclear how exactly the immune system reacts to the SARS-CoV-2 infection. COVID-19 patients' specific and neutralizing anti-bodies can be detectable as early as a few days post-infection. In some cases, however, antibodies were not detected until three to four weeks after the onset of symptoms [10-12]. The main immunogenic parts of the SARS-CoV-2 are the Nucleocapsid protein and the Spike glycoprotein, which induce the immune system to elicit the protective neutralizing antibodies [13,14]. Spike protein is also a key target for developing effective vaccines against SARS-CoV-2 [15], as it is responsible for attaching to the host receptor "Angiotensin-Converting Enzyme 2" for recognition and facilitating viral entrance into the host cell [16]. The presence of undiscovered COVID-19 cases in the population may cause confusion among health officials; on one hand, they may be infectious and capable of transmitting the virus to healthy people [17,18]. On the other hand, detection and measuring the COVID-19 antibodies in the blood will be useful for optimizing the immune status, and will support the immunization campaigns to be more effective and the vaccine doses administered on adequate time [19-21]. As a result, monitoring antibody seropositivity for immunoglobulin G (IgG) and other immunoglobulins will reveal the percentage of previously infected people as well as the level of societal immunity [16,22]. Enzyme-Linked Immunosorbent Assay (ELISA) is a serological test used to identify and/or quantify numerous compounds in serum samples, including antibodies [23, 24]. It is determined by the antigen-antibody complex's development. By measuring the absorbance of a color spectrum generated by a chromogen, the attachment of labelled antibodies to the target material will be observed. The amount of targeted material in the samples is reflected in the measured absorbance. The ELISA technique was been utilized to detect and quantify specific COVID-19 IgG antibodies in human serum samples in this study. This epidemiology study aimed to investigate the prevalence of IgG antibodies to SARS-CoV-2 among the employees of the Royal Scientific Society (RSS) and the whole campus, as a marker of past infections. The RSS is one of Jordan's and the Middle East's largest applied research institutions. Employees in this scientific community were at high risk to be infected with COVID-19. From the start of the pandemic, RSS followed the Ministry of Health's general health and safety precautions and the decision stemming from the law of defense to protect its personnel and the general public. RSS also played a key part in Jordan's fight against COVID-19, deploying their specialist laboratories in a variety of sectors to keep work going and lessen the effects of quarantine. Hence, finding prior infected persons in this community using COVID-19 specific IgG antibody testing was assisting in determining the level of immunization of employees against this virus in this institution. This research study was carried out during the pandemic's second wave, when the national vaccination program was still in its early stages.

Materials & Methods

Study Community

The study conducted is a cross-sectional serological testing study aimed to investigate seropositivity for SARS-CoV-2 among the employees of a scientific institution in Amman, Jordan. The majority of RSS employees are university graduates (Holders of PhD, MSc, BSc in sciences, engineering, financial, business, administration, etch.). The study community also included cleaning, maintenance and catering staff. The RSS employees are from different geographical areas in Jordan; mostly from the middle and north governorates. The variance in educational level and geographical distribution could affect the level of awareness or the degree of applying the precautionary measures among the community, and consequently the level of exposure to the virus. Moreover, social distancing and the number of active cases may also vary from one community to another.

Collection of Blood Samples

Drawing of blood samples was carried out in the medical center of Princess Sumaya University for Technology (Amman-Jordan). A medical doctor and well-trained nurses were commissioned to collect the blood samples. Employees have been contacted over phone to attend at specific time in order to organize the blood drawing without any crowdedness. A consent form was obtained from all employees before blood sampling, and the participants were well informed about the study goals. In addition, all participants were informed about the results of analysis through text messages over their mobile phones.

Samples were collected using venipuncture attached to a red-cap blood tubes. Up to 5 mL of blood was collected from each participant in order to obtain enough amount of serum. All

precautionary safety measures were followed according to the instructions of the local health authorities to control COVID-19 infection and decrease the possibility of transmission; Personal Protective Equipment "PPE" were available all the time, social distancing, periodical disinfecting to the hands and the area of blood sampling. Besides, all employees with disease clinical signs or history of close contact with COVID-19 patients were excluded from the study.

Processing of Blood Samples

All samples were processed at the laboratories of RSS Biosafety and Biosecurity Centre. To avoid the introduction of any analytical bias due to sample preparation, all samples were kept at room temperature to clot for 30 to 60 minutes. Blood samples were centrifuged for 5 minutes (4000 ×g, 25°C) within three hours after blood collection; this step is essential to separate the serum from the clotted blood. Serum samples were harvested to a sterile collection tubes and left at -20°C until subsequent analyses [25].

Qualitative ELISA Assay

Presence or absence of the human anti-COVID-19 IgG antibody in the collected serum samples was tested using EDITM Novel Coronavirus COVID-19 IgG ELISA kit (Epitope Diagnostics, Inc. CA, USA) according to the manufacturer's instructions. Briefly, each serum sample was diluted 1:100 with the sample diluent then loaded to the coated 96-well plate along with the positive and negative controls. The plate was covered and incubated at room temperature for 30 minutes. After aspirating the content and washing steps, the HRP labeled Anti-hIgG Tracer Antibody was added to each well and incubated at room temperature for 30 minutes. Other washing steps were applied, and then the HRP substrate was added, followed by incubation for 20 minutes at room temperature. After this step, the color of positive samples started turn to the blue color. Stop solution was applied immediately to the wells, which let the wells turn to yellow color. The absorbance was read at 450 nm, within 10 minutes of adding the stop solution, using Thermo Scientific™ Multiskan™ GO Microplate Spectrophotometer (Thermo Fisher Scientific, MA, USA). The absorbance reads were exported to an excel sheet to calculate the positive and negative cut off points for each run. Any sample with absorbance higher than the positive cut off point was considered positive for the presence of human anti-COVID-19 IgG antibody.

Quantitative ELISA Assay

All serum samples revealed positive for the presence of human anti-COVID-19 IgG antibody in the qualitative assay, were subjected to quantitative ELISA assay. This was done to quantify the level of human SARS-CoV-2 Ig total using Human SARS-CoV-2 Spike (trimer) Ig Total ELISA kit (Invitrogen, Thermo Fisher Scientific, MA, USA) according to the manufacturer's instructions. Briefly, samples were diluted to 1:75,000 before loading them to the coated wells. Seven standards were loaded to the wells with concentrations from 62.5 to 4000 units/mL, in order to create the standard curve and calculate the antibodies concentration in the samples. The steps of the assay were carried out according to the kit's manual. The absorbance was measured at 450 nm using Thermo Scientific™ Multiskan™ GO Microplate Spectrophotometer (Thermo Fisher Scientific, MA, USA). The reads were exported to excel sheet to create the standard curve and calculate the level of human SARS-CoV-2 Ig total using the generated equation.

Statistical Analysis

The SPSS and OpenEpi softwares were used to statistically analyze the study's data. The effect of these variables on the seroprevalence of COVID-19 Antibodies was examined using the Chi-square test and the One-Way ANOVA test for categorical variables (gender and age) [26]. The population's unequal distribution across gender and age categories is a shortcoming of the statistical analysis used in this study. Since this was a cross-sectional study with a specific demographic in mind, the researchers could not control the distribution of participants in each group.

Patient and Public Involvement

The patient involvement was indirect. The results of both qualitative and quantitative ELISA assays were disseminated to the patients via text messages reached to their phones or verbally. There was no public involvement in this study.

Results

Survey Components

Blood samples were collected between January 1st and February 4th, 2021 from 937 individuals. The distribution of the timing of the blood draws is shown in Figure (1). Participants were interviewed by trained interviewers using a questionnaire that collected information on demographics, social determinants of health, underlying comorbidities or allergies, whether they are taking any kind of drugs, history of COVID-19 PCR test, history of COVID-19 related symptoms during the previous months, and presence of COVID-19 confirmed cases among family, friends, or coworkers.

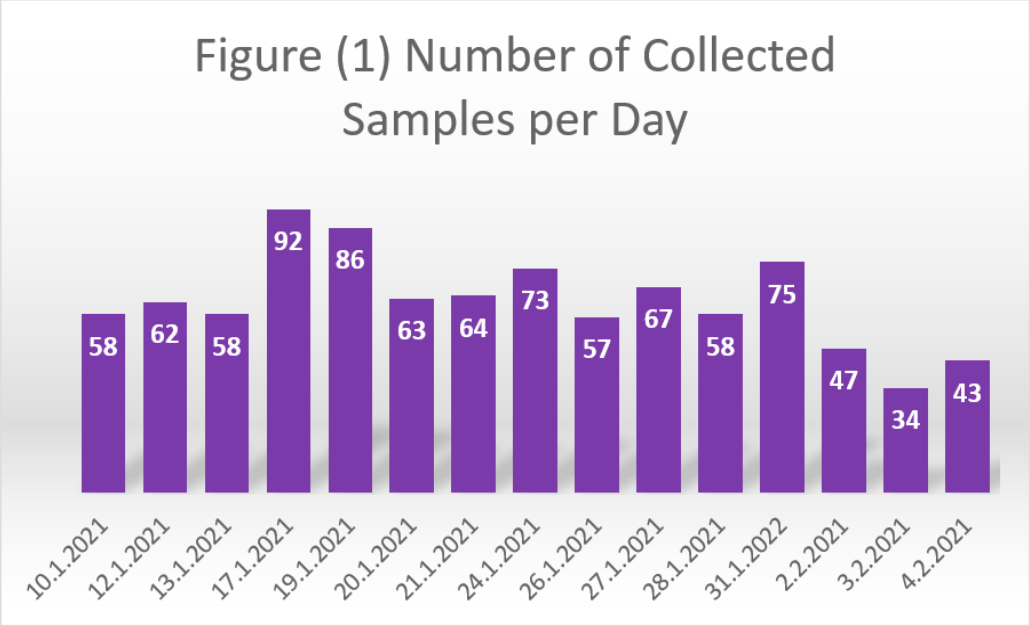

Figure 1: Distribution of the timing of the blood draws between January 1st and February 4th, 2021.

The percentage of participated women in the survey was lower than that for men (31% and 69%, respectively). There is no significant difference in the aspect of prevalence of COVID-19 specific antibodies be-tween the two genders ($P > 0.05$) (Results not shown) 17.3 % for men, and 8.95% for women. The employees were classified to four groups according to their ages. The majority of employees were within the age group (15-47 years) as shown in Table (1). The majority of seropositive samples were located within age group 15-47 (young) ($P < 0.05$). The seroprevalence between age groups were significantly different ($P < 0.05$) even the size of groups was not evenly distributed.

Table 1: Age distribution of the 937 participants from RSS.

Age Group (Year)	Total Number	%	Positive Ab	Positive (%)
Pediatrics (≤ 14)	0	NA	0	NA
Young (15-47)	647	69%	175	72%
Middle-aged (48-64)	277	30%	66	28%
Elderly ≥ 65	13	1%	2	<1%
Total	937		243	

Results of Qualitative ELISA Assay

All serum samples were adequate for testing. Out of the 937 tested participants, 243 serum samples showed seropositivity for the SARS-CoV-2 IgG antibodies. Number of seropositive samples that were not diagnosed previously via PCR was 121 (49.79%) out of total 243 seropositive samples. In contrast, only 26 samples (2.77%) from the previously confirmed positive via PCR test were seronegative for COVID-19 antibodies. Interestingly, 95 (13.83%) out of 687 employees who had no history of infection with SARS-CoV-2 and were not in close contact with COVID-19 patients have antibodies against SARS-CoV-2 as shown in Figure (2).

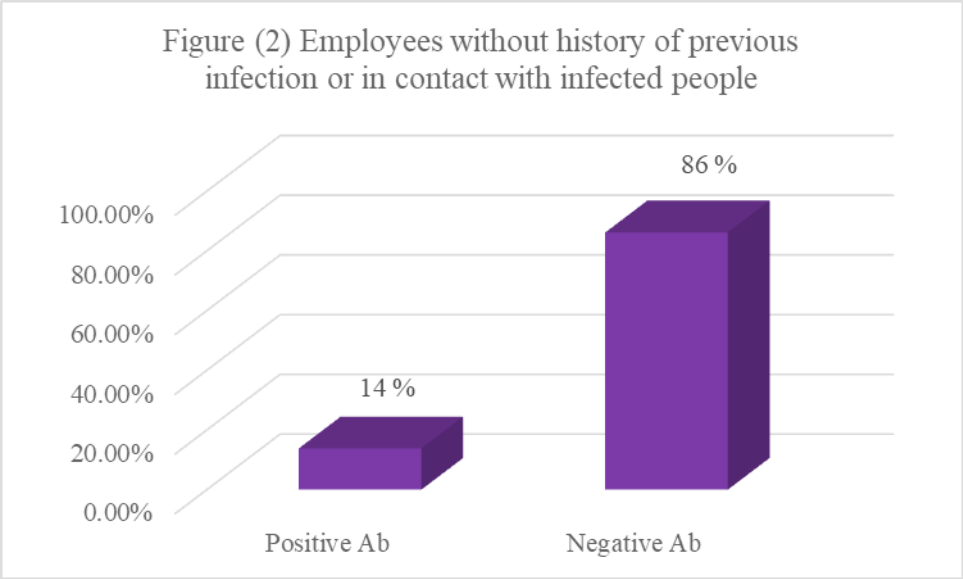

Figure 2: Seroprevalence of IgG antibody among RSS employees without any history of COVID-19 diagnosis or COVID-19 confirmed cases among family, friends, or coworkers.

Among the 179 individuals who reported to be in close contact with infected COVID-19 patients, 90 employees tested positive for SARS-CoV-2-specific antibodies (Figure 3).

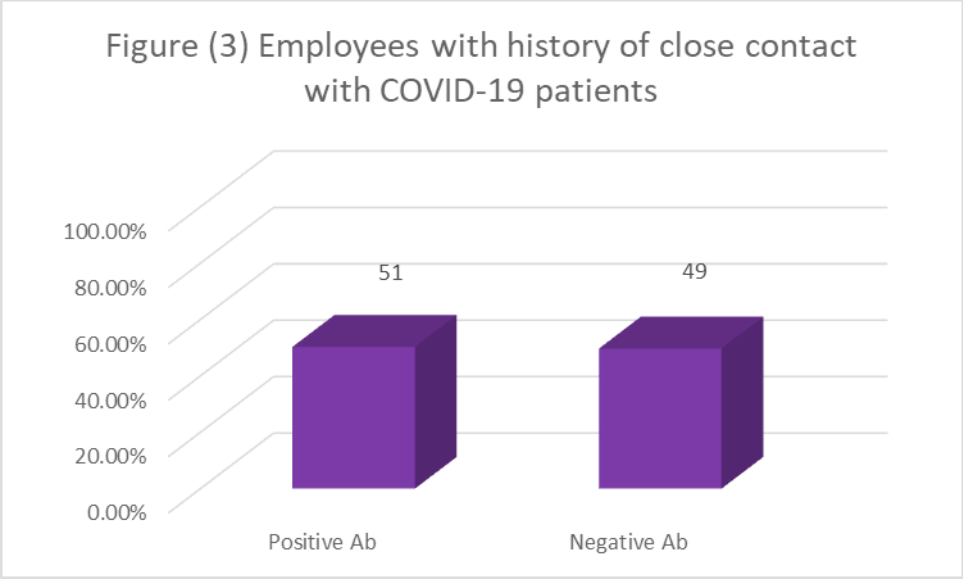

Figure 3: Seroprevalence of IgG antibody among RSS employees with a history of COVID-19 confirmed cases among family, friends, or coworkers.

The number of individuals with underlying comorbidities, diseases affect the immune system, or allergy were 201. Out of those 201 employees, 45 persons (22%) tested positive for SARS-CoV-2 IgG antibody, as shown in Figure (4).

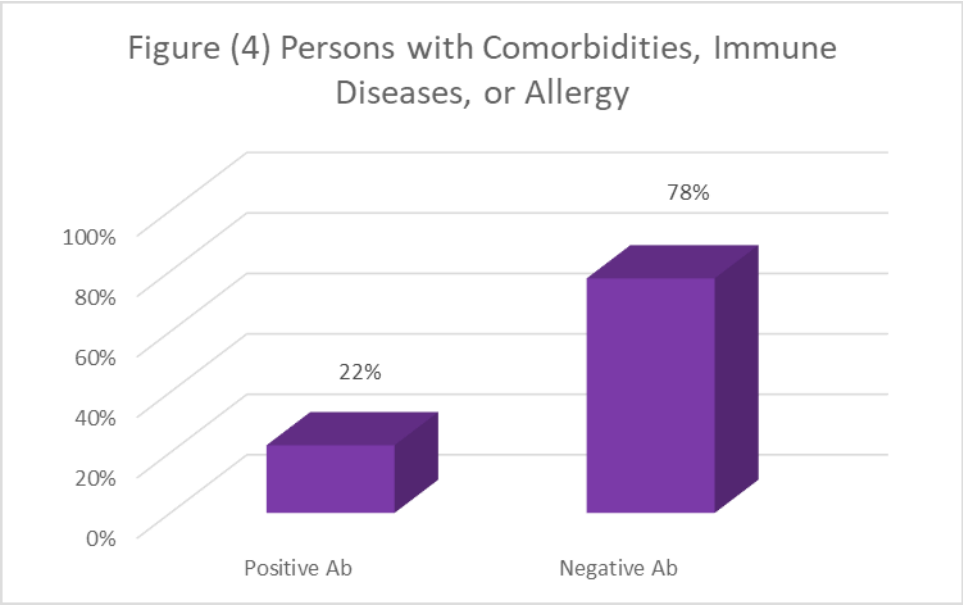

Figure 4: Seroprevalence of IgG antibody among RSS employees with a history of comorbidities, diseases affect the immune system, or allergy.

Five of the study's participants received their first dose of COVID-19 vaccine one or two weeks before blood sampling. SARS-CoV-2 IgG antibodies were not found in any of the sera of those five individuals. According to prior research [19,20], the average time for IgG levels to be found in blood is 14 days after the first dose. This could explain why the IgG antibodies could not be detected in the five vaccinated participants.

Results of Quantitative ELISA Assay

All seropositive serum samples (243) were subjected to the quantitative ELISA assay. The SARS-CoV-2 Ig total antibodies titer was determined for only 133 serum samples. This might be due to the following reasons: The antibody titer for the majority of the other samples was most likely under the limit of detection for the kit. Besides, the samples underwent several freeze-thaw cycles and were highly diluted during the quantitative assay (1:75,000). On the other hand, the antibody titer for most serum samples was between 200-500 units/ml. For few samples, the antibody titer exceeded the 2000 units/ml. Those employees with a high titer of SARS-CoV-2 Ig total antibodies suffered from severe COVID-19 disease symptoms and some of them needed a long recovery period. It is important to emphasize that the level of antibodies in blood changes over time. Therefore, the antibody titer in the tested serum samples reflects the level of antibodies at the time of blood drawing only. Taking into consideration the time between the onset of the infection and the time of blood drawing.

Discussion

SARS-CoV-2 infection in asymptomatic or untested individuals may be undetected by case-based and syndromic surveillance, leaving the general incidence of prior infection unknown. Case-based and syndromic surveillance can be supplemented with serological surveillance. SARS-CoV-2, the virus that causes coronavirus disease 2019 (COVID-19), is still circulating in several Jordanian communities. Despite the importance of case-based and syndromic surveillance in tracking the pandemic, these approaches rely on people being tested or reporting a COVID-19 like disease. An adjunctive strategy for estimating the prevalence of prior infection in a population is to use serologic testing to detect the presence of SARS-CoV-2 antibodies.

To the best of our knowledge, this is the first SARS-CoV-2 seroprevalence study to be conducted in a scientific community in Jordan. The estimated seroprevalence of SARS-CoV-2 IgG antibodies was (25.93%) with 243 positive cases. Among them, only 121 had a a PCR-confirmed case. Based on these results, the number of undiagnosed cases among the RSS employees was estimated to be significantly higher than the number of confirmed cases based on PCR testing.

Moreover, this study focused only on the detection of SARS-CoV-2 antibodies in the target community. Antibodies to SARS-CoV-2 had previously been documented to diminish or even disappear in patients with mild COVID-19 symptoms, which may have contributed to the inability to detect antibodies in a few previously positive cases among RSS employees (26 individuals) [27,28]. Furthermore, it is critical to understand that cellular immunity may play a role in SARS-CoV-2 reinfection immunity [29,30]. Therefore, it is important to conduct further research into cellular immunity. When compared to symptomatic persons in the early stages of recovery, earlier research have shown that asymptomatic individuals have a lower immune response to SARS-CoV-2 infection and a higher percentage of asymptomatic individuals become seronegative. The decrease in neutralizing antibody levels could have ramifications for immunity strategies and serological surveys [11,31].

This study was conducted on subjects without any signs of respiratory or viral illness at the time of blood drawing. In addition, the qualitative and quantitative assays were conducted using different ELISA kits; as the kits targeting different types of antibodies, and have different level of sensitivities, as well as different procedures of testing (e.g., dilution step and the used equations for analysis). This could explain the reasons for not being able to quantify all the positive seroprevalence samples. It was clearly proved that the ELISA assays could vary in the matter of sensitivity, specificity, target Ag or Ab, and the technique itself (e.g., indirect, capture, or sandwich approach) [11,32].

The findings in this study are subject to at least four limitations. First, this was a single-institution study, which may have influenced our results. Second, our statistical power was limited due to the small sample size. Third, because the COVID-19 pandemic is still occurring, many of the available research findings that have been mentioned may be premature. Fourth, the IgM antibody was not tested. Even though a serum is negative for IgG antibodies, it may include a specific IgM antibody; the interpretation of these results will most likely be influenced by IgM quantification.

Conclusions & Recommendations

RSS has used PCR assays to test, track, and trace the epidemic since the beginning. In this scenario, there are few undetected missing cases, according to popular thinking. Despite this stringent strategy of detecting all positive cases regardless of symptoms, a considerable proportion of undiagnosed cases remained unidentified. The findings of this study revealed that there was a reasonably high frequency of personnel in RSS who had a positive SARS-CoV-2 antibody status. According to this study, the true number of RSS employees infected with SARS-CoV-2 far outnumbers the PCR-confirmed cases. The characteristics of SARS-CoV-2, particularly the high proportion of infected individuals who are asymptomatic or have only mild symptoms, and the high transmission rate, may be linked to the missed undiagnosed cases.

Although several seroprevalence studies have been undertaken in Jordan [33-35], this is the first to quantitatively examine antibody levels in a scientific community during the COVID-19 pandemic. Serial evaluation of SARS-CoV-2 IgG antibody status is anticipated to reveal risk variables for COVID-19 susceptibility and disease transmission mechanisms. These findings should be interpreted with caution, as there is still a paucity of evidence about the role of antibodies present after recovery from COVID-19 in the development of immunity against subsequent infections. The emergence of several variants of concern, as well as the implementation of COVID-19 immunization, will have an impact on population immunity. As reports of a second infection continue to stream in, herd immunity in the context of COVID-19 is a point of contention. This underlines the importance of maintaining health precautions and risk-mitigation behaviors in order to keep the outbreak under control.

Seroprevalence data are crucial for determining the pandemic's scope and distribution, as well as predicting the likelihood and timing of future waves of recrudescence. It can also deal with public health issues including the safety of stay-at-home orders or school closures, as well as analyses of alternative therapies and interventions [36].

To monitor the SARS-CoV-2 seroprevalence in Jordan and inform policymakers on the efficacy of their surveillance system, it is advised that population-based seroprevalence studies be conducted on a regular basis.

Acknowledgments

Great thanking and appreciation to the Royal Scientific Society (Amman-Jordan) for providing the necessary facilities and a conducive environment for this research. Special thanks to the Royal Scientific Society's Health, Safety, and Work Environment Department for assisting with the blood sample events, as well as Princess Sumaya University for Technology (Amman, Jordan) for providing a designated blood sampling area at the University Medical Center. Finally, we would like to express our gratitude to the Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) GmbH for their ongoing support of our programs, as well as the German Federal Ministry for Economic Cooperation and Development (BMZ) for funding this research.

Funding

The study was implemented by the Royal Scientific Society (RSS) of Jordan with the financial support of the German Federal Ministry for Economic Cooperation and Development (BMZ), Agreement No. (81266031). The contents of this manuscript are the sole responsibility of RSS and does not necessarily reflect the views of the BMZ.

Author Contributions

NH: Designing the study, supervising the activities of the study, managing and supervising the technical and financial aspects of the study, interpretation of the results and writing the manuscript. MR: Coordinating blood-sampling events, conducting the qualitative and quantitative ELISA assays in addition to the statistical analysis, interpretation of the results, and writing the manuscript. GZ and AA: Taking demographical data, processing of blood samples, and conducting ELISA Assays. BH: Contributing in designing the study, and interpretation of the results.

Conflict of Interest

The authors have declared that no competing interests exist.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethical Approval

The study was approved by the RSS Executive Committee (Decision No. 3/2022).

Informed Consent

The manuscript does not contain any individual person's data in any form.

References

1. Lin Z, Yang R, Li K, Yi G, Li Z, et al., (2020) Establishment of age group classification for risk stratification in glioma patients. BMC Neurol 20: 310.
2. Zhu N, Zhang D, Wang W, Li X, Yang B, et al., (2020) A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med 382:727-733.
3. Rossi GA, Sacco O, Mancino E, Cristiani L, Midulla F, et al., (2020) Differences and similarities between SARS-CoV and SARS-CoV-2: spike receptor-binding domain recognition and host cell infection with support of cellular serine proteases. Infection 48:665-669.
4. Rabaan AA, Al-Ahmed SH, Haque S, Sah R, Tiwari R, et al., (2020) SARS-CoV-2, SARS-CoV, and MERS-COV: A comparative overview. Infez Med 28:174-184.
5. Chakraborty C, Sharma AR, Sharma G, Bhattacharya M, Lee SS (2020) SARS-CoV-2 causing pneumonia-associated respiratory disorder (COVID-19): diagnos-tic and proposed therapeutic options. Eur Rev Med Pharmacol Sci 24:4016-4026.
6. WHO (2021) WHO Coronavirus (COVID-19) Dashboard. 2021 3 December.
7. Alqutob R, Al Nsour M, Tarawneh MR, Ajlouni M, Khader Y, et al., (2020) COVID-19 Crisis in Jordan: Response, Scenarios, Strategies, and Recommendations. JMIR Public Health Surveill 6: e19332.
8. Al-Tammemi AB, Tarhini Z, Akour A (2021) A swaying between successive pandemic waves and pandemic fatigue: Where does Jordan stand? Ann Med Surg (Lond) 65:102298.
9. WHO (2021) WHO Coronavirus (COVID-19) Dashboard. 2021 4 December.
10. Xiao AT, Gao C, Zhang S (2020) Profile of specific antibodies to SARS-CoV-2: The first report. J Infect 81: 147-178.
11. Zhao J, Yuan Q, Wang H, Liu W, Liao X, et al., (2020) Antibody Responses to SARS-CoV-2 in Patients With Novel Coronavirus Disease 2019. Clin Infect Dis 71:2027-2034.
12. Combadiere B (2020) [Adaptive immunity against SARS-CoV-2]. Med Sci (Paris) 36:908-913.
13. Jiang S, Hillyer C, Du L (2020) Neutralizing Antibodies against SARS-CoV-2 and Other Human Coronaviruses. Trends Immunol 41:355-359.
14. Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, et al., (2020) Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. Cell 181:281-292 e6.
15. Bonanni P, Canton R, Gill D, Halfon P, Liebert UG, et al., (2021) The Role of Serology Testing to Strengthen Vaccination Initiatives and Policies for COVID-19 in Europe. COVID 1: 20-38.
16. Grant R, Dub T, Andrianou X, Nohynek H, Wilder-Smith A, et al., (2021) SARS-CoV-2 population-based seroprevalence studies in Europe: a scoping review. BMJ Open 11: e045425.
17. Bai Y, Yao L, Wei T, et al., (2020) Presumed Asymptomatic Carrier Transmission of COVID-19. JAMA 323: 1406-1407.
18. Gao WJ, Li LM (2020) [Advances on presymptomatic or asymptomatic carrier transmission of COVID-19]. Zhonghua Liu Xing Bing Xue Za Zhi 41: 485-488.
19. Amanat F, Stadlbauer D, Strohmeier S, Nguyen TH, Chromikova V, et al., (2020) A serological assay to detect SARS-CoV-2 seroconversion in humans. Nat Med 26:1033-1036.
20. Melgoza-Gonzalez EA, Hinojosa-Trujillo D, Reséndiz-Sandoval M, Mata-Haro V, Hernández-Valenzuela S, et al., (2022) Analysis of IgG, IgA and IgM antibodies against SARS-CoV-2 spike protein S1 in convalescent and vaccinated patients with the Pfizer-BioNTech and CanSinoBio vaccines. Transbound Emerg Dis 69:e734-e745.
21. Bonanni P, Canton R, Gill D, Halfon P, Liebert UG, et al., (2021) The Role of Serology Testing to Strengthen Vaccination Initiatives and Policies for COVID-19 in Europe. COVID 1: 20-38.
22. Bobrovitz N, Arora RK, Cao C, Boucher E, Liu M, et al., (2021) Global seroprevalence of SARS-CoV-2 antibodies: A systematic review and me-ta-analysis. PLoS One 16:e0252617.
23. Shah K. Maghsoudlou P (2016) Enzyme-linked immunosorbent assay (ELISA): the basics. Br J Hosp Med (Lond) 77: C98-101.

24. Alhajj M, Zubair M, Farhana A (2023) Enzyme Linked Immunosorbent Assay, in StatPearls. 2021: Treasure Island (FL).

25. Tuck MK, Chan DW, Chia D, Godwin AK, Grizzle WE, et al., (2009) Standard operating procedures for serum and plasma collection: early detection research network consensus statement standard operating procedure integration working group. J Proteome Res 8:113-117.

26. Kurtzke JF (1966) On statistical testing of prevalence studies. J Chronic Dis 19:909-922.

27. Yang OO, Ibarrondo FJ (2020) Loss of Anti-SARS-CoV-2 Antibodies in Mild Covid-19. Reply. N Engl J Med, 383:1697-1698.

28. Ibarrondo FJ, Fulcher JA, Goodman-Meza D, Elliott J, Hofmann C, et al., (2020) Rapid Decay of Anti-SARS-CoV-2 Antibodies in Persons with Mild Covid-19. N Engl J Med 383:1085-1087.

29. Mahase E (2020) Covid-19: Two antibody tests are “highly specific” but vary in sensitivity, evaluations find. BMJ 369:m2066.

30. Zheng J, Deng Y, Zhao Z, Mao B, Lu M, et al., (2022) Characterization of SARS-CoV-2-specific humoral immunity and its potential applications and therapeutic prospects. Cell Mol Immunol 19:150-157.

31. Wu LP, Wang NC, Chang YH, Tian XY, Na DY, et al., (2007) Duration of antibody responses after severe acute respiratory syndrome. Emerg Infect Dis 13:1562-1564.

32. Zhang Z, Wang X, Wei X, Zheng SW, Lenhart BJ, et al., (2021) Multiplex quantitative detection of SARS-CoV-2 specific IgG and IgM antibodies based on DNA-assisted nanopore sensing. Biosens Bioelectron 181:113134.

33. Bellizzi S, Alsawalha L, Ali SS, Sharkas G, Muthu N, et al., (2021) A three-phase population based sero-epidemiological study: Assessing the trend in preva-lence of SARS-CoV-2 during COVID-19 pandemic in Jordan. One Health 13:100292.

34. Sughayer MA, Mansour A, Nuirat AA, Souan L, Ghanem M, et al., (2021) Dramatic rise in seroprevalence rates of SARS-CoV-2 antibodies among healthy blood donors: The evolution of a pandemic. Int J Infect Dis 107:116-120.

35. Elnasser Z, Obeidat H, Amarin Z, Alrabadi N, Jaradat A, et al., (2021) Prevalence of COVID-19 among blood donors: The Jordan University of Science and Technology experience. Medicine (Baltimore) 100: e27537.

36. Bryant JE, Azman AS, Ferrari MJ, Arnold BF, Boni MF, et al., (2020) Serology for SARS-CoV-2: Apprehensions, opportunities, and the path forward. Sci Immunol 5: eabc6347.