



# A three-phase population based sero-epidemiological study: Assessing the trend in prevalence of SARS-CoV-2 during COVID-19 pandemic in Jordan

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## ABSTRACT

The evolution of the COVID-19 pandemic in Jordan during the first 10 months of the epidemic was peculiar and can be easily categorized in three different phases: a first period featuring a very low number of reported cases, a second period with exponential growth from August with up to 8000 cases on the 18th November 2020, and a third phase with steady and progressive decline of the epidemiological curve. With the aim of better determine the entity of the population exposed to SARS-CoV-2, the Jordan Ministry of Health with the support of the WHO launched three rounds of the nationwide sero-prevalence survey. Using population proportionate to size (PPS) methodology, around 5000 individuals were selected from all Jordan governorates. Blood samples were collected from all participants and ELISA assays for total IgM, IgG antibodies to COVID-19 were used for testing at the National Public Health Laboratory. Results revealed that seroprevalence dramatically increased over time, with only a tiny fraction of seropositive individuals in August (0.3%), to increase up to more than 20-fold in October (7.0%) and to reach one-third of the overall population exposed by the end of 2020 (34.2%). While non age-specific trends were detected in infection rates across different age categories, in all three rounds of the seroprevalence study two out of three positive participants did not report any sign and/or symptom compatible with COVID-19. The serial cross-sectional surveys experience in Jordan allowed to gain additional insights of the epidemic over time in combination with context-specific aspects like adherence to public health and social measures (PHSM). On the other hand, such findings would be helpful for planning of public health mitigation measures like vaccinations and tailored restriction policies.

## 1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first reported in China in December 2019 and rapidly spread throughout the world [1]. While global estimates rely on notifications of symptomatic cases of respective illness related to COVID-19, the actual infection rates in the community is not accurately reflected due to the large majority of infected individuals being pauci-symptomatic or asymptomatic.

With almost 300,000 confirmed cases and 4000 deaths by the very beginning of January 2021, Jordan represented about 6.0% of the total

confirmed cases and 3.0% of the total number of deaths in the WHO Eastern Mediterranean Region (EMR), which in turn included 7.0% of the total cases globally reported. Over the same period, Jordan also ranked sixth among the most hit countries in EMR, following Iran, Iraq, Pakistan, Morocco and the Kingdom of Saudi Arabia [2].

To note how countries in EMR have a historical unique vulnerability to emerging infectious diseases. Specifically, the peculiar geographical location, which falls under various global migratory bird flight paths, and the annual mass gatherings during Islamic pilgrimage, Hajj, have led to the rise and rapid transmission of diseases with zoonotic origins like Avian Influenza A, pandemic H1N1/2009 virus, and MERS-CoV

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[3].

The evolution of the COVID-19 transmission in Jordan during the first 10 months of the pandemic can be uniquely categorized according to the three different phases of public health measures implemented in the country (Fig. 1): a) a first phase since last week of February 2020, where the government applied strict control measures, including comprehensive lockdown and full closure of all borders, thus flattening the epidemiological curve and prolonging sporadic transmission [4]; b) a second phase features progressive easing of restrictions with an exponential increase of cases up to 8000 particularly on a single day during November 2020, placing Jordan in the list of countries that had highest number of cases in the world, and; c) a third phase that showed a steady and progressive decline of the epidemiological curve with an average of 2000 cases per day over the last week of December 2020 [5].

The above-mentioned phases were captured through the epidemiological surveillance of severe symptomatic confirmed COVID-19 cases and might hide additional peculiar features of the pandemic in Jordan. It is crucial to recognize an infected person at an early stage to cut the transmission of COVID-19, although in reality they do not often require medication attention or contribute to rapid spread of the infection. But it is not sufficient to rely only on confirmed cases of COVID-19 cases detected by polymerase chain reaction (PCR) for control measures, as these tests often rule out asymptomatic and pre-symptomatic cases [6]. In order to overcome this challenge, A quantitative, population-based sero-epidemiological survey can better determine the entity of the population exposed to SARS-CoV-2 by revealing the proportion of the population with antibodies against the virus, to generate data on immunity status of population and to implement containment measures accordingly.

While most of the previous serological surveys of SARS-CoV-2 relied on small or non-random sampling of study participants (e.g. focusing on health-care workers or blood donors) [7–10], only few have been conducted on national-scale and with precise estimates of seroprevalence by age group in the general population [11,12].

In collaboration with the World Health Organization, the Jordan Ministry of Health launched three rounds of nationwide, population-based studies to quantify the extent of SARS-CoV-2 circulation in relation to the different phases of the transmission throughout the country.

## 2. Methods

### 2.1. Unity studies

The serological surveys for SARS-CoV-2 were conducted as a part of global WHO Unity Studies initiative to understand multiple unknown characteristics related to the COVID-19 pandemic; specifically, these studies aimed to rapidly gather data and define disease epidemiological pattern and guide control measures by ascertaining the cumulative population immunity.

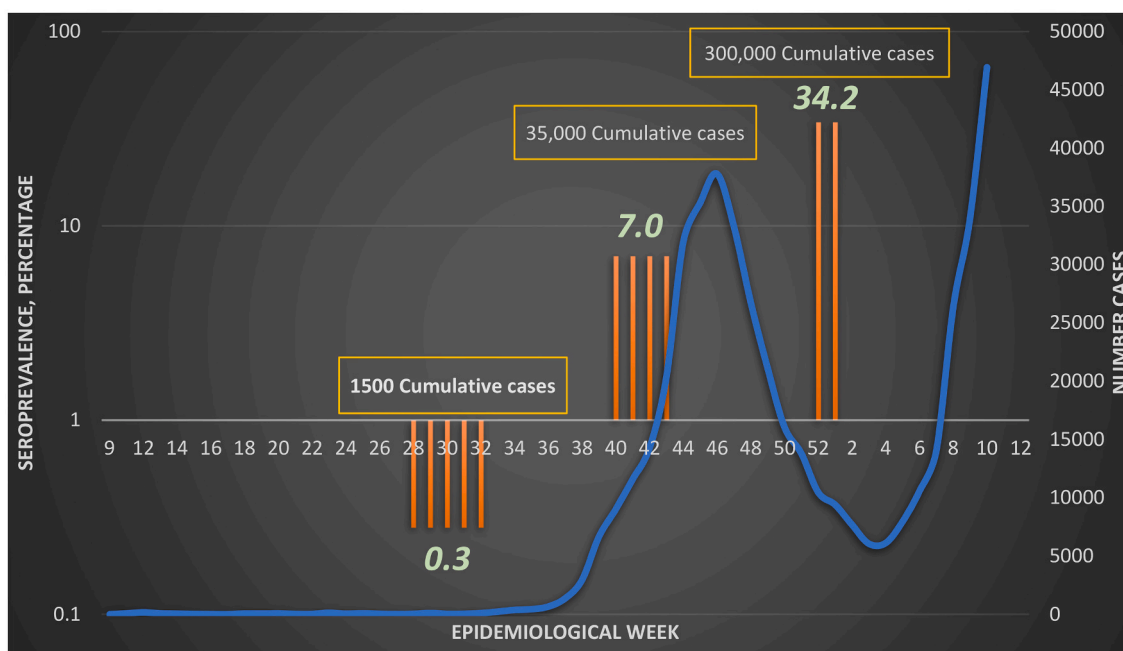
Three independent repeated cross-sectional population-based studies were carried out during the months of August 2020, October 2020, and January 2021, with each survey lasting one month of field implementation.

### 2.2. Study population and sample size

Population in three regions of Jordan (North, middle, and South) and each region is composed of 4 governorates (Table 1). All governorates were included in the study and the study population was assigned using

**Table 1**  
Categorization of Jordan across the three Regions and relative Governorates with sampling proportional to size.

Region	Governorate	% population	First Survey	Second Survey	Third Survey
North	Irbid	18.6	831	831	910
	Ajloon	1.8	80	80	88
	Jarash	2.5	112	112	122
	Mafraq	5.8	259	259	284
Middle	Amman	42	1877	1877	2056
	Zarka	14.3	639	639	700
	Balka	5.2	232	232	255
	Madaba	2	89	89	100
South	Karak	3.3	147	147	165
	Tafilla	1	45	45	50
	Ma'an	1.5	67	67	75
	Aqaba	2	89	89	90
Total		100	4469	4469	4895



**Fig. 1.** Weekly total COVID-19 reported cases during the first 10 months of the epidemic in Jordan, proportion of participants positive to IgM/IgG for SARS-CoV-2 and cumulative number of COVID-19 reported cases in three different time-periods.

population proportionate to size (PPS) of areas.

Based on estimated prevalence of 3.0% with an acceptable margin of error 0.5% (95.0% confidence level), the final sample size consisted of 4469 persons from 812 household. A household was defined as a group of people (two or more) living in the same residence or as two or more people living together in a domestic residence.

### 2.3. Data collection and selection criteria

Ten field teams were deployed with each team including one epidemiologist, one data collector or public health inspector, and one laboratory technician. The study was based on field home visits to collect data and specimens.

All persons living in the household were invited to participate in the study, including children to ensure the calculation of age-specific attack rates. Multiple time periods of field visits were conducted to ensure the inclusion of working individuals.

Exclusion criteria included the selection of household with less than 2 members willing to participate in the study, refusal to provide informed consent and/or contraindication to venipuncture, residential institutions such as boarding schools, dormitories, hostels and prisons were excluded. Suspected, confirmed acute and prior COVID-19 infection were not excluded from participation in the study.

In each household, participants were questioned to fill questionnaire on demographic information and information on comorbidities. Questions also included on the history of confirmed diagnosis of COVID-19 infection and household contacts with persons with confirmed COVID-19, COVID-19 related symptoms a month before the survey.

### 2.4. Blood sample and processing

Blood samples were collected from all individuals who participated in the study and 2 ml of venous blood (2 ml from the younger participants) was collected from participants by trained laboratory technicians or a trained nurse in a sterile tube with gel; for each biological sample collected, a list with the time of collection, the conditions for transportation and the time of arrival at the study laboratory were recorded. Specimens reached the laboratory within 12 h except those from the southern areas, which reached the laboratory within 72 h.

Serum samples were transported at 4.0 °C except those from those from the southern areas, which were separated from whole blood prior to sending to laboratory frozen at -20 °C. They were subsequently aliquoted and frozen at -80 °C until testing after 10–14 days of collection at the National Public Health Laboratory. Wantai / ELISA assays for total IgM, IgG antibodies to COVID-19 were used for testing at the National Public Health Laboratory.

All procedures involving sample manipulation were carried out in a biosafety cabinet (BSC) at least level 2 (BSL-2). Personals and researchers involved in the sample collection and transportation of specimens were trained for safe handling practices and spill decontamination procedures, including use of PPE.

### 2.5. Ethical considerations

The study protocol was submitted and approved by the Jordan Ministry of Health Institutional Review Board (IRB). A written informed consent was signed by the household participants prior to participation in the study. Similar consent for children under the legal age of consent was obtained from a parent or legal guardian.

The purpose of the investigation was explained to all participants and were informed on the benefit of participation as collected data will help to improve and guide efforts to understand extent of COVID-19 virus infection and may prevent further transmission of the virus.

Participant confidentiality was maintained throughout the investigation. All participants were assigned a study identification number by the investigation team for the labelling of questionnaires and specimens.

The link of this identification number to individuals was maintained by the investigation team and the Ministry of Health and was not disclosed elsewhere.

### 2.6. Statistical analysis

Statistical analysis was conducted using the software STATA v13.1 SE (StataCorp LP, College Station, Texas, USA) [13]. We conducted a descriptive statistical analysis by measuring counts and percentage of individuals testing positive for the IgG against SARS-CoV-2 in all the Jordan areas under study separately for the first, second and third round. Distribution of positivity was also stratified by age group and gender. Additionally, number of individuals with serologic evidence of SARS-CoV-2 was tabulated according to the reporting of signs and/or symptoms attributable to COVID-19 since the onset of the epidemic in Jordan.

## 3. Results

### 3.1. First round of sero-epidemiological study

During the month of August 2020, a total of 1222 households were visited and 1007 met the inclusion criteria, with a mean household participant of 4 individuals and a total of 4704 tested individuals (87.2% participation rate). The Mean age of study population was 29 years old with female representing 56.5% ( $n = 2647$ ) of the total sample ( $n = 4681$ ), which included 13 COVID-19 PCR-positive cases. The overall prevalence was 0.3% while in the same period around 1500 cumulative cases were reported at national level (Fig. 1). The highest proportion of participants with positivity to antibodies was in Jarash governorate (0.9%), in the age group 60–64 (0.8%) and among females (0.3%). While female prevalence (0.3%) was slightly higher than males one (0.2%), asymptomatic individuals showed more than five times seropositivity of symptomatic participants (Table 2).

### 3.2. Second round of sero-epidemiological study

The second round of the seroprevalence survey conducted in the same geographical areas during October 2020 comprised a total of 1043 screened household with 5470 out of 6457 individuals participating in the study (84.0% response rate) and with an average of 5 screened individuals per household. The Mean age of study population was 32 while 54% (2954) of screened individuals were females. This second survey revealed a 7.0% overall prevalence in a time-period when around 35,000 cumulative cases were reported at national level (Fig. 1). Almost 10.0% was the proportion among the 15–19 years old age group and slightly more than 35.0% prevalence in the Ma'an governorate. Also, female individuals showed higher positivity when compared to male counterpart (8.1% versus 5.5%). Asymptomatic individuals accounted for as much as double the prevalence of symptomatic participants to the study (4.7% versus 2.3%) (Table 2).

### 3.3. Third round of sero-epidemiological study

The third round of the seroprevalence survey was conducted in the same geographical areas from 27th December 2020 through 6th January 2021 when about 300,000 cumulative cases were reported at national level. It comprised a total of 840 screened household with 6120 individuals. Out of the total eligible individuals, 5044 participate in the study with response rate of 82%, and with an average number of 6 screened individuals per household.

The mean age of study population was 32 and slightly higher representation of women were present in line with the previous two rounds (Table 2). The overall prevalence of SARS-CoV-2 antibodies reached 34.2% over the third round conducted at the beginning of 2021 (Fig. 1). More than half of participants in Ma'an Governorate revealed positivity

**Table 2**

Total number of positive tests for SARS-CoV-2 IgM/IgG with relative percentage and 95% Confidence Interval by round of survey as well as by Governorate, age-group, gender and presence of symptoms.

Area	First survey		Second survey		Third survey	
	Positive tests	Percentage (95% CI)	Positive tests	Percentage (95% CI)	Positive tests	Percentage (95% CI)
Amman	6	0.3 (0.2–0.4)	168	8.2 (7.9–8.4)	817	38.0 (37.5–38.5)
Ajloon	0	0	1	1.2 (1.1–1.3)	42	41.2 (40.6–41.8)
Aqaba	0	0	4	4.5 (4.3–4.7)	38	36.5 (36.1–36.9)
Balka	2	0.8 (0.6–0.9)	32	8.4 (8.2–8.6)	69	27.1 (26.7–27.5)
Irbid	4	0.4 (0.3–0.5)	18	2.1 (1.9–2.2)	342	35.4 (35.0–35.9)
Jarash	1	0.9 (0.7–1.1)	3	3.2 (3.0–3.3)	42	28.6 (28.2–29.0)
Karak	0	0	27	17.3 (17.0–17.5)	80	45.7 (45.1–46.3)
Ma'an	0	0	20	35.1 (34.7–35.4)	32	53.3 (52.5–54.1)
Madaba	0	0	0	0.0	21	18.6 (18.4–18.9)
Mafraq	0	0	6	2.1 (1.9–2.2)	49	17.2 (16.9–17.4)
Tafila	0	0	0	0.0	24	44.4 (44.0–44.9)
Zarqa	0	0	69	8.6 (8.4–8.8)	167	26.3 (25.9–26.6)
<i>Age-group</i>						
<5		0.0	13	5.2 (5.0–5.4)	32	29.1 (28.8–29.3)
5–9	2	0.4 (0.3–0.5)	34	8.8 (8.5–9.0)	126	38.0 (37.6–38.3)
10–14		0.0	41	6.9 (6.7–7.1)	179	36.3 (36.0–36.6)
15–19	1	0.2 (0.1–0.3)	42	9.6 (9.4–9.9)	201	36.5 (36.1–36.9)
20–29	2	0.4 (0.3–0.5)	50	7.1 (6.9–7.3)	283	31.4 (31.3–31.7)
30–59	6	0.4 (0.3–0.5)	152	6.7 (6.6–6.8)	740	34.8 (34.3–35.2)
60–64	2	0.8 (0.7–1.0)	7	4.5 (4.4–4.6)	91	39.1 (38.6–39.6)
65+		0.0	9	4.4 (4.3–4.5)	71	30.0 (29.5–30.4)
<i>Gender</i>						
Male	5	0.2 (0.1–0.3)	121	5.5 (5.4–5.7)	723	31.9 (31.6–32.5)
Female	8	0.3 (0.2–0.4)	227	8.1 (7.9–8.3)	1000	37.5 (37.1–38.0)
<i>Infection type</i>						
Symptomatic	2	0.1 (0.1–0.2)	114	2.3 (2.2–2.4)	652	45.1 (44.6–45.7)
Asymptomatic	11	0.2 (0.1–0.2)	234	4.7 (4.6–4.8)	1071	29.8 (29.4–30.2)
Total	13	0.3 (0.2–0.4)	348	7.0 (6.8–7.2)	1723	34.2 (33.8–34.6)

and the age groups 5–9 and 60–64 years old as well as the “female” category reached almost 40.0%. Contrary to the previous two surveys, many more participants who showed antibodies in the 2020/21 study showed also at least one symptom compatible with COVID-19 (Table 2).

#### 4. Discussion

This study used three series of cross-sectional surveys to interpret the seroprevalence to SARS-CoV-2 in Jordan, from the early phase of the pandemic to the late 2020 post-peak phase. Seroprevalence dramatically increased over time, with only a tiny fraction of seropositive individuals in August, to increase up to more than 20-fold in October and to reach one-third of the overall population exposed by the end of 2020/beginning of 2021.

Such a trend is an accurate reflection of the evolution of the transmission also in line with government policies and public behaviors: the first case of COVID-19 in Jordan was reported on the 2 March 2020, and the government closed the country's borders on 15 March 2020, in addition to suspending schools, banning public gatherings, and issuing a comprehensive lockdown with implementation of curfew. By mid-August, the COVID-19 transmission in Jordan elevated, with the recording of 20–30 cases per day and toward the end of August, the daily cases were 30–40. This quickly escalated to several hundred and then to several thousand cases a day, in the following months, most likely due to a lack of public compliance with recommendations [14].

Findings from the three phases of sero prevalence survey conducted in different time-periods revealed the importance of testing capacity and testing strategy in Jordan overtime. According to the results of the first survey, considering the Jordan population of around 10 million, around 30,000 individuals were exposed to SARS-CoV-2 in August; this represents around 20 times more than the officially reported 1500 cases reported though the national surveillance. The same proportion can be derived when considering results of the second survey in October, which was around 35,000 cases officially reported, while around 700,000 cases

might have already been exposed. Interestingly, such a ratio halved at the end of 2020 when around 300,000 cumulative cases were reported by the Ministry of Health Surveillance Department and the third round of the seroprevalence study indicated that slightly more than 3,000,000 cases resulted positive for IgM/IgG against the SARS-CoV-2.

To note that the Jordan Government invested importantly on testing over time, from less than 15,000 tests per day in August 2020 to up to 30,000 tests per day during the second half of October and during November when Jordan was hardly hit by a first big wave that reached the peak on the 18 November with almost 8000 cases. Concurrently, the national testing strategy was re-directed during the third quarter of 2020 to reduce random sampling and target more specifically symptomatic individuals. Finally, we cannot underestimate the role of awareness and care-seeking behavior, which might have increased due to risk communication and community engagement campaigns as well as to the perceived burden due to COVID-19 within the society.

The higher proportion of individuals with antibodies against SARS-CoV-2 during the second and the third round in the Governorate of Ma'an is most likely due to the fact that in both surveys the collection of specimens in this area was left to the very last days of fieldwork, thus allowing for more people to get exposed when compared to other Governorates where collections was conducted up to three/four weeks earlier.

The increase in seroprevalence over time, even in an area of presumed low transmission, indicates that seroprevalence studies may serve as important tools to determine the spread of infection in populations where a large majority of the people are asymptomatic. Monitoring the general population through serial serosurveys can detect resurgence, especially when lockdown measures are eased, and enable policy makers to devise strategies for containment of the disease [15].

The survey did not identify any age-specific trends in infection rates across different age categories. While prevalence appeared to increase with age less than 19 years in the second survey, the third round showed a slightly higher prevalence among very young (38.0% in 5/9 years old)



and elderly (39.0% in 60/64 years old). These findings are not in line with previous reports indicating children and adolescent (0–19 years) and older people ( $\geq 60$  years) had lower seroprevalence than the other age groups [11,16–18]. Such results might be attributable to no decreased social mixing in these age groups despite the studies were conducted while schools were closed for months and recommendations to protect the elderly were widespread.

On the other hand, prevalence held constantly higher among females with 0.3%, 8.1% and 37.0% compared to 0.2%, 5.5% and 31.0% in respectively the first, second and third surveys. This is in contrast with other studies where seroprevalence was higher in males than in the female population [19,20]. However, a report around Wuhan city and other places in China revealed that females had greater odds of being IgG positive than males (0.91% and 0.60%, respectively;  $\chi^2 = 17.01$ ,  $P < 10^{-4}$ ), suggesting that females have higher levels of activation of immune cells, leading to higher levels of antibodies against SARS-CoV-2 produced in females than in males [21,22].

As far as symptoms are concerned, in all three rounds of the seroprevalence study two out of three positive participants did not report any sign and/or symptom compatible with COVID-19. This is consistent with a previous similar report conducted in Pakistan where only three of 10 reported any respiratory symptoms, with or without fever [13]. In contrast to these findings, the proportion of asymptomatic infections was reported to be much lower (27.7%, 95% CI 16.4–42.7%) in a meta-analysis [23].

Our study adopted a strong methodology recommended by the World Health Organization, and a strong aspect is also represented by the fact that the Wantai/ELISA assays used to process the specimens reported very high specificity, which is needed especially early in the pandemic or in areas with low transmission in order to accurately measure the seroprevalence.

However, various limitations need to be highlighted. Participation rates to all rounds were below 90% and ranged from slightly more than 87% during the first survey to 82% over the last one. This indicates some sort of volunteer bias, which could have affected the final estimates in either direction. Estimates of seroprevalence will tend to be high if individuals are more likely to accept testing because they think they have been exposed to SARS-CoV-2. On the other hand, a downward bias will occur if exposed individuals avoid testing because they do not want a positive test result [24]. It is also important to note how sensitivity is often lower for individuals with lower antibody titers. Therefore rates of false negatives are expected to be higher among younger individuals [25,26], or individuals with less severe disease [27,28], such as individuals who were recently infected and have not yet mounted an antibody response [29]. Another relevant item to consider is related to the low sensitivity for individuals who were infected long before testing (as antibody titers wane over the weeks and months after infection): this represents an important issue when using seropositivity as a proxy for the cumulative incidence of infection in a population [24].

In conclusion, the three rounds of the nation-wide seroepidemiological survey in Jordan showed an exponential growth of the individuals exposed to the SARS-CoV-2 along the different phases of the epidemic. The latest round of the three surveys revealed the potential impact of the devastating epidemic wave that took place between October and November, and that led to around one out of three individuals living in Jordan to be exposed to the coronavirus. We believe that the serial cross-sectional surveys experience at national level has a two-fold fruitful finalities: it allows to gain additional insights of the epidemic over time in combination with context-specific aspects like adherence to public health and social measures (PHSM), and allows for planning of public health mitigation measures like vaccinations and tailored restriction policies.

The seroprevalence survey three-round experience is another reminder of the potential zoonotic disease's role in public health as well as of the existing limitations for detecting and preventing the emergence of a Public Health Emergency of International Concern (PHEIC) through

real-time surveillance. To this end, Jordan has been greatly investing on multi-sectoral planning (human, animal, and environmental health sectors), communication, and collaboration between humans, animals, and the environment sectors to advance the One Health approach.

### Authors' contribution

SB, LA, WH, MCP and NMO conceptualized the manuscript. SB and SSA engaged in data curation and conducted the statistical analysis. SB, GS and NM and MG worked on the methodology. GS, WH, MCP, and NMO oversaw the project administration and validated the findings. SB, LA and NM wrote the first draft of the manuscript. SSA, GS, MG, WH, MCP and NMO reviewed and edited the first draft of the manuscript. SB, LA, SSA and NM revised and finalized the manuscript. All authors agreed on the final version of the manuscript.

### Declaration of Competing Interest

No competing interests.

### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.onehlt.2021.100292>.

### References

- [1] World Health Organization, Coronavirus disease (COVID-19) situation reports [cited 2020 Aug 10], <https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports>, 2020.
- [2] <http://www.emro.who.int/health-topics/corona-virus/index.html>.
- [3] K.A. Kheirallah, A.H. Al-Mistarehi, L. Alsawalha, Z. Hijazeen, H. Mahrous, S. Sheikali, S. Al-Ramini, M. Maayeh, R. Dodeen, M. Farajeh, N. Masadeh, A. Alemam, J. Alsulaiman, D. Samhour, Prioritizing zoonotic diseases utilizing the One Health approach: Jordan's experience, *One Health* 13 (2021 May 1) 100262, <https://doi.org/10.1016/j.onehlt.2021.100262>. PMID: 34027008; PMCID: PMC8121978.
- [4] M. Al-Tamimi, R. Qaisieh, R.E. Tadros, M. Shalabi, A. Alhasoun, M.M. Kilani, Successful flattening of COVID-19 epidemiological curve in Jordan, *J. Glob. Health* 10 (2) (2020 Dec) 020361, <https://doi.org/10.7189/jogh.10.020361>. PMID: 33282214; PMCID: PMC7688063.
- [5] Ministry of Health, the official website of the Jordanian Ministry of Health | Coronavirus disease. [moh.gov.jo](http://moh.gov.jo).
- [6] L.R. Inbaraj, C.E. George, S. Chandrasingh, Seroprevalence of COVID-19 infection in rural district of South India: a population based sero-epidemiological study, *PLoS One* (2021), <https://doi.org/10.1371/journal.pone.0249247>.
- [7] H. Salje, C.T. Kiem, N. Lefrancq, Estimating the burden of SARS-CoV-2 in France, *Science*. (2020), <https://doi.org/10.1126/science.abc3517>.
- [8] A. Bryan, G. Pepper, M.H. Wener, Performance characteristics of the Abbott Architect SARS-CoV-2 IgG assay and seroprevalence in Boise, Idaho, *J. Clin. Microbiol.* (2020), <https://doi.org/10.1128/JCM.00941-20>.
- [9] N. Sood, P. Simon, P. Ebner, Seroprevalence of SARS-CoV-2-specific antibodies among adults in Los Angeles County, California, on April 10–11, 2020, *JAMA*. (2020), <https://doi.org/10.1001/jama.2020.8279> published online May 18.
- [10] X. Wu, B. Fu, L. Chen, Y. Feng, Serological tests facilitate identification of asymptomatic SARS-CoV-2 infection in Wuhan, China, *J. Med. Virol.* (2020), <https://doi.org/10.1002/jmv.25904>.
- [11] M. Pollán, B. Pérez-Gómez, R. Pastor-Barriuso, J. Oteo, M.A. Hernán, M. Pérez-Olmeda, J.L. Sanmartín, A. Fernández-García, I. Cruz, N. Fernández de Larrea, M. Molina, F. Rodríguez-Cabrera, M. Martín, P. Merino-Amador, J. León Paniagua, J.F. Muñoz-Montalvo, F. Blanco, R. Yotti, ENE-COVID Study Group, Prevalence of SARS-CoV-2 in Spain (ENE-COVID): a nationwide, population-based seroepidemiological study, *Lancet* 396 (10250) (2020 Aug 22) 535–544, [https://doi.org/10.1016/S0140-6736\(20\)31483-5](https://doi.org/10.1016/S0140-6736(20)31483-5). Epub 2020 Jul 6. PMID: 32645347; PMCID: PMC7336131.
- [12] A.J.D., C.G. Victora, SARS-CoV-2 antibody prevalence in Brazil: results from two successive nationwide serological household surveys, *Lancet Glob. Health* 8 (11) (2020 Nov) e1390–e1398, [https://doi.org/10.1016/S2214-109X\(20\)30387-9](https://doi.org/10.1016/S2214-109X(20)30387-9). Epub 2020 Sep 23. PMID: 32979314; PMCID: PMC7511212.
- [13] Stata Corp, *Statistical Software: Release 13 SE [Computer Program]*, College Station, TX, Stata Corp, 2013.
- [14] E. Alnazy, O.M. Khraisat, A.M. Al-Bashairah, C.L. Bryant, Anxiety, depression, stress, fear and social support during COVID-19 pandemic among Jordanian healthcare workers, *PLoS One* 16 (3) (2021 Mar 12) e0247679, <https://doi.org/10.1371/journal.pone.0247679>. PMID: 33711026; PMCID: PMC7954309.
- [15] M.I. Nisar, N. Ansari, F. Khalid, M. Amin, H. Shahbaz, A. Hotwani, N. Rehman, S. Pugh, U. Mehmood, A. Rizvi, A. Memon, Z. Ahmed, A. Ahmed, J. Iqbal, A. F. Saleem, U.B. Aamir, D.B. Larremore, B. Fosdick, F. Jehan, Serial population-

- based serosurveys for COVID-19 in two neighbourhoods of Karachi, Pakistan, *Int. J. Infect. Dis.* 106 (2021 Mar 15) 176–182, <https://doi.org/10.1016/j.ijid.2021.03.040>. Epub ahead of print. PMID: 33737137.
- [16] A.R. Alsuwaidi, F.I. Al Hosani, S. Al Memari, H. Narchi, L. Abdel Wareth, H. Kamal, M. Al Ketbi, D. Al Baloushi, A. Elfateh, A. Khudair, S. Al Mazrouei, H. S. AlHumaidan, N. Alghaihi, K. Afsh, N. Al Kaabi, B. Altrabulsi, M. Jones, S. Shaban, M. Sheek-Hussein, T. Zoubeidi, Seroprevalence of COVID-19 infection in the Emirate of Abu Dhabi, United Arab Emirates: a population-based cross-sectional study, *Int. J. Epidemiol.* (2021 Apr 24) dyab077, <https://doi.org/10.1093/ije/dyab077>. Epub ahead of print. PMID: 33893483.
- [17] S. Stringhini, A. Wisniak, G. Piumatti, A.S. Azman, S.A. Lauer, H. Baysson, D. De Ridder, D. Petrovic, S. Schrepf, K. Marcus, S. Yerly, I. Arm Vernez, O. Keiser, S. Hurst, K.M. Posfay-Barbe, D. Trono, D. Pittet, L. Gétaz, F. Chappuis, I. Eckerle, N. Vuilleumier, B. Meyer, A. Flahault, L. Kaiser, I. Guessous, Seroprevalence of anti-SARS-CoV-2 IgG antibodies in Geneva, Switzerland (SEROCoV-POP): a population-based study, *Lancet* 396 (10247) (2020 Aug 1) 313–319, [https://doi.org/10.1016/S0140-6736\(20\)31304-0](https://doi.org/10.1016/S0140-6736(20)31304-0). Epub 2020 Jun 11. PMID: 32534626; PMCID: PMC7289564.
- [18] P.C. Hallal, F.P. Hartwig, B.L. Horta, M.F. Silveira, C.J. Struchiner, L.P. Vidaletti, N.A. Neumann, L.C. Pellanda, O.A. Dellagostin, M.N. Burattini, G.D. Victora, A.M. B. Menezes, F.C. Barros, A.J.D. Barros, C.G. Victora, SARS-CoV-2 antibody prevalence in Brazil: results from two successive nationwide serological household surveys, *Lancet Glob. Health* 8 (11) (2020 Nov) e1390–e1398, [https://doi.org/10.1016/S2214-109X\(20\)30387-9](https://doi.org/10.1016/S2214-109X(20)30387-9). Epub 2020 Sep 23. PMID: 32979314; PMCID: PMC7511212.
- [19] E.S. Rosenberg, J.M. Tesoriero, E.M. Rosenthal, R. Chung, M.A. Barranco, L. M. Styer, M.M. Parker, S.Y. John Leung, J.E. Morne, D. Greene, D.R. Holtgrave, D. Hoefler, J. Kumar, T. Udo, B. Hutton, H.A. Zucker, Cumulative incidence and diagnosis of SARS-CoV-2 infection in New York, *Ann. Epidemiol.* 48 (2020 Aug) 23–29, e4, <https://doi.org/10.1016/j.annepidem.2020.06.004>. Epub 2020 Jun 17. PMID: 32648546; PMCID: PMC7297691.
- [20] L. Amorim Filho, C.L. Szwarcwald, S.O.G. Mateos, A.C.M.P. Leon, R.A. Medronho, V.G. Veloso, J.I.F. Lopes, L.C.M.S. Porto, A. Chieppe, G.L. Werneck, Grupo Hemorio de Pesquisa em Covid-19, Seroprevalence of anti-SARS-CoV-2 among blood donors in Rio de Janeiro, Brazil, *Rev. Saude Publica* 54 (2020) 69, <https://doi.org/10.11606/s1518-8787.2020054002643>. Epub 2020 Jul 6. PMID: 32638883; PMCID: PMC7334006.
- [21] S. Duan, M. Zhou, W. Zhang, J. Shen, R. Qi, X. Qin, H. Yu, C. Zhou, Q. Hu, X.J. Yu, Seroprevalence and asymptomatic carrier status of SARS-CoV-2 in Wuhan City and other places of China, *PLoS Negl. Trop. Dis.* 15 (1) (2021 Jan 7) e0008975, <https://doi.org/10.1371/journal.pntd.0008975>. PMID: 33411805; PMCID: PMC7790301.
- [22] P. Conti, A. Younes, Coronavirus COV-19/SARS-CoV-2 affects women less than men: clinical response to viral infection, *J. Biol. Regul. Homeost. Agents March-April*;34 (2) (2020) 339–343, <https://doi.org/10.23812/Editorial-Conti-3>. PMID: 32253888.
- [23] J. He, Y. Guo, R. Mao, J. Zhang, Proportion of asymptomatic coronavirus disease 2019 (COVID-19): a systematic review and meta-analysis, *J. Med. Virol.* 93 (2021) 820–830.
- [24] E.K. Accorsi, X. Qiu, E. Rumpfer, L. Kennedy-Shaffer, R. Kahn, K. Joshi, E. Goldstein, M.J. Stensrud, R. Niehus, M. Cevik, M. Lipsitch, How to detect and reduce potential sources of biases in studies of SARS-CoV-2 and COVID-19, *Eur. J. Epidemiol.* 36 (2) (2021 Feb) 179–196, <https://doi.org/10.1007/s10654-021-00727-7>. Epub 2021 Feb 25. PMID: 33634345; PMCID: PMC7906244.
- [25] S.F. Lumley, J. Wei, D. O'Donnell, N.E. Stoesser, P.C. Matthews, A. Howarth, et al., The duration, dynamics and determinants of SARS-CoV-2 antibody responses in individual healthcare workers, *Infect. Dis. (except HIV/AIDS)*. medRxiv (2020) CD013652, <https://doi.org/10.1101/2020.11.02.20224824>.
- [26] Y. Liu, B. Mao, S. Liang, J.-W. Yang, H.-W. Lu, Y.-H. Chai, et al., Association between age and clinical characteristics and outcomes of COVID-19, *Eur. Respir. J.* (2020), <https://doi.org/10.1183/13993003.01112-2020>.
- [27] G.A. Poland, I.G. Ovsyannikova, R.B. Kennedy, SARS-CoV-2 immunity: review and applications to phase 3 vaccine candidates, *Lancet.* 396 (2020) 1595–1606, [https://doi.org/10.1016/S0140-6736\(20\)32137-1](https://doi.org/10.1016/S0140-6736(20)32137-1).
- [28] K.L. Lynch, J.D. Whitman, N.P. Lacaienta, E.W. Beckerdite, S.A. Kastner, B.R. Shy, et al., Magnitude and kinetics of anti-severe acute respiratory syndrome coronavirus 2 antibody responses and their relationship to disease severity, *Clin. Infect. Dis.* 72 (2) (2021) 301–308, <https://doi.org/10.1093/cid/ciaa979>. PMID: 33501951; PMCID: PMC7454426.
- [29] N. Sethuraman, S.S. Jeremiah, A. Ryo, Interpreting diagnostic tests for SARS-CoV-2, *JAMA.* (2020), <https://doi.org/10.1001/jama.2020.8259>.