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SARS-CoV-2 seroprevalence in Aden, Yemen: a population-based study

Abdulla Salem Bin-Ghouth a,⁎, Sheikh Al-Shoteri b, Nuha Mahmoud d, Altaf Musani d, Nasser Mohsen Baom c, Ali Ahmed Al-Waleedi e, Evans Buliva f, Eman AbdelKreem Aly e, Jeremias Domingos Naiene g, Rosa Crestani e, Mikiko Senga e, Amal Barakat e, Lubna Al-Ariqi e, Khaled Zein Al-Sakkaf b, Abeer Shae f, Najib Thabit d, Ahmed Murshed d, Samuel Omara d

a College of Medicine, Hadhramout University, Mukalla, Yemen
b Aden University, Yemen
c Ministry of Public Health and Population, Yemen
d World Health Organization Country Office, Yemen
e World Health Organization Eastern Mediterranean Regional Office, Cairo, Egypt

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Abstract

Background: In Yemen, initial surveillance of coronavirus disease 2019 (COVID-19) focused primarily on patients with symptoms or severe disease. The full spectrum of the disease remains unclear. To the best of the authors’ knowledge, this is the first seroprevalence study performed in Yemen.

Methods: This cross-sectional investigation included 2001 participants from all age groups from four districts in Aden, southern Yemen. A multi-stage sampling method was used. Data were collected using a well-structured questionnaire, and blood samples were taken. Healgen COVID-19 IgG/IgM Rapid Diagnostic Test (RDT) Cassettes were used in all participants. All positive RDTs and 14% of negative RDTs underwent enzyme-linked immunosorbent assay (ELISA) testing (WANTAI SARS-CoV-2 Ab ELISA Kit) for confirmation.

Results: In total, 549 of 2001 participants were RDT positive and confirmed by ELISA, giving a prevalence of COVID-19 of 27.4%. The prevalence of immunoglobulin G was 25%. The prevalence of asymptomatic COVID-19 in the entire study group was 7.9%. The highest prevalence was observed in Al-Mansurah district (33.4%). Regarding sociodemographic factors, the prevalence of COVID-19 was significantly higher among females, housewives and subjects with a history of contact with a COVID-19 patient: 32%, 31% and 39%, respectively.

Conclusion: This study found high prevalence of COVID-19 in the study population. Household transmission was common.

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Introduction

Coronavirus disease 2019 (COVID-19) spread rapidly around the world after the first case was reported in Wuhan, China in December 2019. In Yemen, as in other resource- limited countries (e.g. countries in Africa) (Nkengasong and Mankoula, 2020), the epidemiology of COVID 19 is uncertain due to weak healthcare systems; inadequate surveillance and laboratory capacity; scarcity of public health resources; and limited financial means, treatment and vaccines. It has been reported elsewhere that uncertainty and underestimation of the true number of cases may be due to insufficient testing (Gupta et al., 2020).

Initial surveillance focused primarily on patients with symptoms or severe disease. The full spectrum of COVID-19, including the proportion of mild or asymptomatic cases that do not require medical attention, remains unclear (World Health Organization, 2020a).

As the great majority of cases of COVID-19 in Yemen have been reported from hospital-based surveillance, estimates of the case fatality rate and other epidemiological parameters are likely to be
lower than reality. Once the full spectrum of cases of COVID-19 are included in the denominator (i.e. inclusion of asymptomatic or mild cases), estimates are likely be be more accurate. It has been reported elsewhere that measuring case fatality rates and projecting the number of deaths depends on the estimated total number of infections (Li et al., 2020; Onder et al., 2020; Spychalski et al., 2020; Wu et al., 2020), but in the absence of seroprevalence surveys, this is estimated by multiplying the number of cases by certain factors. However, the magnitude of these factors is highly uncertain.

Up to 22 November 2020 (1 week before this survey was conducted), there had been 2073 confirmed cases of COVID-19 and 605 related deaths in Yemen (case fatality rate 29%) (World Health Organization, 2020b), with presumed community transmission occurring in large cities such as Aden, particularly in May 2020. To the authors’ knowledge, no previous seroprevalence studies have been undertaken in Yemen to inform epidemic parameters. As such, this study aimed to measure the seroprevalence of antibodies to severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) in a community sample from Aden, and more specifically to measure the seroprevalence of antibodies to SARS-CoV-2 in the general population by sex and age group, in order to ascertain cumulative population immunity and to estimate the proportion of asymptomatic cases.

Methods

Study design and participants

The methodology was developed according to the World Health Organization protocol for population-level SARS-CoV-2 antibody testing. This cross-sectional, community-based study was conducted in November–December 2020. The sample size was calculated to be a total of 2000 participants from all age groups. Multi-stage sampling was used. The study was undertaken in Aden governorate in southern Yemen (population 997,308). Specifically, four of the eight districts in Aden were selected at random (two from high-incidence districts and two from low-incidence districts), and two locations (zones) were selected at random from each district. In each location, one direction was selected at random from the central point, and all the houses in this direction were selected. In each house, one male and one female were selected at random from all household members to be in the sample.

Data collection

Data were collected using a well-structured questionnaire covering demographic and clinical variables. The questionnaire was digitized using KOBO toolbox application with data validation rules, skip logic and quality control measures integrated to minimize data entry errors. The forms were deployed through mobile phones. This allowed for efficient data collection with real-time data transmission directly from the field. The electronic questionnaire for each household was assigned a unique ID that was later used to match the respondent field records of rapid diagnostic test (RDT) results, and the confirmatory enzyme-linked immunosorbent assay (ELISA) results from the laboratory. Both laboratory results and field records were transmitted electronically using KOBO toolbox to facilitate analysis.

Laboratory methods

A serum sample was collected from each participant, and screened in the field for the presence of SARS-CoV-2 antibodies by trained laboratory staff using RDT. For each biological sample collected, time of collection, conditions for transportation and time of arrival at the central public health laboratory (CPHL) in Aden were recorded. Specimens reached the laboratory within 2–3 h of collection. Serum was separated from whole blood and shipped at 4°C, or frozen to -20°C or lower (-80°C) and shipped on dry ice. If the specimens were not likely to reach the laboratory within 72 h, specimens were frozen, preferably at -80°C, and shipped on dry ice.

The RDT (USA) used in this study was the Healgen COVID-19 IgG/IgM Cassette, and WANTAI SARS-CoV-2 Ab ELISA (China) was used for confirmatory testing; this ELISA is highly specific and sensitive. All patients with positive RDT results underwent confirmatory ELISA testing, and 14% of patients with negative RDT results also underwent ELISA testing.

Statistical analysis

Data were entered in Excel (Microsoft Corp., Redmond, WA, USA), and then imported into EpiData (Denmark), SPSS Version 23 (IBM Corp., Armonk, NY, USA) and STATA 16 (StataCorp., College Station, TX, USA) for descriptive and inferential statistical analysis. Confidence intervals (CI) and odds ratios were used to test differences in prevalence rates. Student’s t-test and Chi-squared test were used, as appropriate. Multi-variate analysis was used to assess the impact of social and risk factors for COVID-19. A significance level of 0.05 was used.

Ethical approval

Ethical approval was obtained from the Research Ethics Committee (REC) of the Faculty of Medicine of Aden University (REC-78-2020, 20 August 2020). The REC approved the research proposal and found it to be compliant with the International Conference of Harmonization.

Results

Description of the participants

The research team visited 1138 houses; generally, one or two individuals were included from each household, although three or four individuals were included from 218 households (19%). In total, 2006 questionnaires were returned from the field survey; however, five of these questionnaires were duplicates and were excluded from the analysis. As such, the data for 2001 subjects were included in this analysis. The study population included subjects of both genders and all age groups from four of the eight districts of Aden. The mean age of participants was 33.8 years (standard deviation 17.1), the median age was 31 years (interquartile range 25), and the age range was 1 month to 90 years. Figure 1a shows the population pyramid of the study population. The number of children aged <5 years and 5–15 years in the study group was not representative of the sizes of these age groups in the population of Aden. Inclusion of children in research is of high ethical concern which limits obtaining representative numbers from this group.

RDT validation and final sample included in the analysis

RDTs were performed in the field to detect SARS-CoV-2 antibodies. In total, 610 subjects had positive RDT results. All RDT-positive samples and 14% of the RDT-negative samples were sent to CPHL for validation by ELISA. In total, 549 samples were confirmed to be true-positive cases by ELISA. Table 1 shows the prevalence of COVID-19 by district, and compared this with prevalence by RDT. The prevalence of SARS-CoV-2 antibodies based on RDT and confirmed by ELISA was 27.4% (95% CI 25.6–29.3%). Distribution of the final sample selected for analysis (549 subjects) by age and sex is presented in Figure 1b.
Prevalence of SARS-CoV-2 IgG antibodies

The prevalence rates of different antibodies using RDT and ELISA are presented in Table 2. The prevalence of IgG was 25% (95% CI 23.2–26.9%), whereas the prevalence of IgM was just 0.2% (95% CI 0.1–0.4%).

Prevalence of asymptomatic COVID-19

The prevalence of asymptomatic COVID-19 among the study group in Aden was 7.8% (157/2001). The proportion of symptomatic cases among the RDT-positive subjects in this survey was 71.4% (reported at least one symptom related to COVID-19), and the remaining 28.6% of RDT-positive subjects were asymptomatic (Table 3).

Seroprevalence of SARS-CoV-2 infection by sociodemographic characteristics and risk factors

The prevalence of SARS-CoV-2 antibodies was 27.5% (95% CI 25.6–29.3%). Although the highest prevalence rates were seen among subjects aged 15–29 years (28.2%) and 45–64 years (28.1%), this was not significantly different compared with other age groups. Unfortunately, the prevalence of COVID-19 was found to be high among children aged <5 years (23%). Regarding gender, the prevalence of COVID-19 in females (32%) was significantly higher compared with males (23%) (95% CI 1.28–1.91). The highest prevalence was observed in Al-Mansurah district (33%), followed by Dar-Saad district (28.3%) and Sera district (27.7%). Khour-Maksar district had lower prevalence compared with the other districts (20.7%), and this difference was significant. Regarding occupation, the highest prevalence of COVID-19 was observed among health workers (31.6%), housewives (31%), students (29.7%), and school...

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**Table 1**
Prevalence of severe acute respiratory syndrome coronavirus-2 antibodies in Aden based on rapid diagnostic testing (RDT) and confirmed by enzyme-linked immunosorbent assay (ELISA).

| Location       | Total RDT | RDT positive | RDT and ELISA positive (as verified by ELISA) |
|----------------|-----------|--------------|-----------------------------------------------|
| Al Mansura     | 500       | 183 (37%)    | 165 (33%)                                      |
| Crafter        | 498       | 152 (31%)    | 139 (27.7%)                                   |
| Dar Sad        | 502       | 160 (32%)    | 142 (28.3%)                                   |
| Khur Maksar    | 501       | 115 (23%)    | 103 (20.7%)                                   |
| Total          | 2001      | 610 (30%)    | 549 (27.4%) (25.6–29.3%)                     |

**Table 2**
Seroprevalence of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) antibodies, Aden, December 2020.

| Participants with SARS-CoV-2 positive test | Prevalence point estimate (%) | 95% CI       |
|-------------------------------------------|-------------------------------|-------------|
| IgG                                       | 500                           | 25%         | 23.2–26.9% |
| IgM                                       | 4                             | 0.2%        | 0.1–0.4%   |
| Both IgG + IgM                            | 45                            | 2.3%        | 1.7–2.9%   |
| Total                                     | 549                           | 27.4%       | 25.6–29.3% |

Ig, immunoglobulin; CI, confidence interval.

**Table 3**
Proportion of asymptomatic cases among all severe acute respiratory syndrome coronavirus-2-positive subjects.

|                        | RDT-positive subjects | Proportion | 95% CI     |
|------------------------|-----------------------|------------|------------|
| Symptomatic            | 392                   | 71.4%      | 67.4–75%   |
| Asymptomatic           | 157                   | 28.6%      | 25–32.1%   |
| Total                  | 549                   | 100%       |            |

RDT, rapid diagnostic test; CI, confidence interval.
Table 4

| Age group (years) | Number | Sex | District | Nationality | Occupation | History of contact with COVID-19 | Reference group |
|-------------------|--------|-----|----------|-------------|------------|---------------------------------|----------------|
| <5                | 3      | 12  | 13       | 23%         | 1          | Reference group                 |                |
| 5–14              | 61     | 191 | 252      | 24.2%       | 1.05       | 0.28–1.59                       |                |
| 15–29             | 176    | 448 | 624      | 28.2%       | 1.22       | 0.35–4.81                       |                |
| 30–44             | 155    | 398 | 553      | 28%         | 1.21       | 0.35–4.87                       |                |
| 45–64             | 126    | 322 | 448      | 28.1%       | 1.22       | 0.35–4.81                       |                |
| ≥65               | 28     | 83  | 111      | 25.2%       | 1.09       | 0.28–4.37                       |                |

Sex
- Female: 309, 655, 964, 32%, 1.39, 1.28–
- Male: 240, 797, 1037, 23%, 1.91

District
- Khourmaksar: 104, 397, 501, 20.7%, 1
- Sera (Creater): 138, 360, 498, 27.7%, 1.33
- Al-Mansurah: 165, 335, 500, 33%, 1.59
- Dar-Saad: 142, 360, 502, 28.3%, 1.36

Nationality
- Yemeni: 548, 1450, 1998, 274%, 0.82
- Non-Yemeni: 1, 2, 3, 33.3%, 14.6

Occupation
- Unemployed: 92, 273, 365, 25.2%, 1
- Armed forces: 38, 118, 156, 24.3%, 0.96
- Health worker: 19, 41, 60, 31.6%, 1.25
- School teacher/academic: 13, 33, 46, 28.2%, 1.12
- Other workers: 66, 231, 297, 22.2%, 0.91
- Retired: 14, 59, 73, 19%, 0.78
- Housewife: 213, 472, 685, 31%, 1.96
- Student: 91, 215, 306, 29.7%, 1.22

History of contact with a COVID-19 patient
- Yes: 104, 160, 264, 39%, 1.56
- No: 445, 1292, 1737, 25%, 2.47

COVID-19, coronavirus disease 2019.

* Significant difference.

b Thirteen children aged <5 years were excluded from the occupation analysis, so the number of participants with a SARS-CoV-2-positive test was 546 and the total number of participants with a SARS-CoV-2-negative test was 1442. The total number of participants for the occupation analysis was 1888.

Table 5

| Table 5 | Results of multi-variate analysis of the role of sociodemographic factors in coronavirus disease 2019 (COVID-19). |
|---------|---------------------------------------------------------------------------------------------------------------|
| Chi-squared | P-value                                                                                      |
| Age group             | 3.760 | 0.584                                                                                 |
| Sex                    | 9.449 | 0.002*                                                                               |
| District               | 16.165 | 0.000*                                                                               |
| Nationality            | 0.084 | 0.772                                                                                 |
| Occupation             | 8.644 | 0.279                                                                                 |
| Contact with COVID-19  | 14.228 | 0.000*                                                                               |

* Significant, P<0.05.

Seroprevalence rates for anti-SARS-CoV-2 antibodies in different parts of the world have shown disparity. The rate is low in the developed world, with reports of 5% in Spain (Pollán et al., 2020) and 6.9% in the USA (Havers et al., 2020). In a recent systematic review, SARS-CoV-2 seroprevalence in the general population varied from 0.37% to 22.1%, with a pooled estimate of 3.38% (95% CI 3.05–3.72%); at regional level, seroprevalence varied from 1.45% (95% CI 0.95–1.94%) in South America to 5.27% (95% CI 3.97–6.57%) in Northern Europe (Rostami et al., 2021).

This study found that SARS-CoV-2 antibodies were present among 27.4% of the study population in Aden. Although these results are in contrast with findings from Western populations, they are consistent with population studies in developing countries [22% for Iran and 22–33% for India (Bhattacharyya et al., 2020; Rostami et al., 2021; Shakiha et al., 2020), and 34% for Pakistan (Zaidi et al., 2021)]; this high prevalence rate may be explained by the lack of public health measures in Aden.

In terms of specific antibodies, the prevalence of IgG was 25% in this study, which was more than the reported prevalence in Brazil (8.3%) (Borges et al., 2020) and Italy (4.5%) (Alessi et al., 2020).

Methodological differences may explain the high prevalence of SARS-CoV-2 antibodies in Aden, as most other published studies have targeted either specific groups or specific locations, such as asymptomatic individuals at markets in the study from Brazil. In the present study, a house-to-house survey was used to target a broad group of people, and the research team had no background information about cases of infection or disease history. It is difficult...
to eliminate selection bias in fieldwork, so this may have overestimated the seroprevalence rate. The major disadvantage is that resulting estimates will often not reflect the true seroprevalence rate in the underlying population due to selection bias (Shook-Sa et al., 2020). The prevalence of asymptomatic cases of COVID-19 in this study (8.5%) was consistent with prevalence rates reported in the studies mentioned above.

The level of SARS-CoV-2 antibodies in the study population may not reflect the level of herd immunity. No vaccines were available in Yemen at the time of this survey and the level of susceptibility remained high. The point at which the proportion of susceptible individuals falls below the threshold needed for transmission is known as the ‘herd immunity threshold’ (Anderson and May, 1985). Above the herd immunity threshold, those who remain susceptible may benefit indirectly from infection. (Zaidi et al., 2021) concluded in their study in Pakistan that the seroprevalence of SARS-CoV-2 antibodies was 36%, which was below the herd immunity threshold (at least 60–70% in the general population). Moreover, it is not known if previous infection with SARS-CoV-2 in Aden provides protection against new variants.

The prevalence of SARS-CoV-2 antibodies was high in all age groups in this study, and increased from children to adolescents and adults. The same conclusions were reported in the USA. SARS-CoV-2 seroprevalence was lower in older adults compared with younger adults across nearly all jurisdictions (Anand et al., 2020; Stringhini et al., 2020). With endemic coronaviruses, seroprevalence typically increases through childhood into early adulthood (Huang et al., 2020), and a few studies of SARS-CoV-2 have shown seroprevalence to be lower in children and adolescents (age <18 years) compared with young adults (Pollán et al., 2020).

Regarding sex, seroprevalence of SARS-CoV-2 antibodies was found to be higher in females than males in this study. This could suggest greater risk of household transmission. In a seroprevalence study in the USA, (Bajema et al., 2021) found no consistent differences between men and women. Seroprevalence was higher in women in some states, but higher in men in other states. High household transmission was observed in this study, as high prevalence rates were reported in housewives and many subjects with SARS-CoV-2 antibodies had been in contact with a COVID-19 patient. SARS-CoV-2 is more transmissible in households (Jing et al., 2020); household contacts and those travelling with a case were at higher risk of infection (Bi et al., 2020). Cheng et al. (2020) concluded in their study in Taiwan that the attack rate was higher among family contacts.

In this study, approximately 72% of RDT-positive cases had at least one symptom related to COVID-19. A systematic review by Buitrago-Garcia et al. (2020) of 79 studies in a range of different settings found that 20% (95% CI 17–25%) of subjects with SARS-CoV-2 infection remained asymptomatic during follow-up; and in seven studies of defined populations screened for SARS-CoV-2 and then followed, 31% (95% CI 26–37%) of subjects remained asymptomatic. These findings are consistent with the present results.

**Limitations**

While all RDT-positive samples (n=549) were tested by ELISA in this study, only 14% of RDT-negative samples were tested by ELISA. Subjects who were RDT positive and ELISA negative were excluded from the analysis. In addition, subjects who were ELISA positive and RDT negative were excluded from the analysis, so this method may have underestimated the true prevalence rate. Another limitation of this study is that measurements were only taken from a small random population in Aden. In future studies, it is important to test all subjects with ELISA and to increase the sample size.

**Conclusions**

Although high prevalence of SARS-CoV-2 antibodies was reported, this does not suggest herd immunity as >70% of all participants were still susceptible. As another wave of COVID-19 is anticipated in Yemen, there is a need to increase population immunity by introducing COVID-19 vaccines. Females, housewives and subjects with a history of contact with a COVID-19 patient were among the highest infected groups in the study sample. As well as the household setting, health and education settings are sites of possible exposure due to lack of social distancing.

**Conflict of interest statement**

None declared.

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**Author contributions**

Bin-Ghouth AS: principal investigator, managed all stages of research from proposal conceptualization to study design, prepared questionnaire, trained data collectors, supervised fieldwork, formal analysis including maintenance of research data (software code) for data interpretation and initial use and subsequent re-use, and wrote first and final drafts of report.

Al-Shoteri SH: prepared laboratory methodology, trained laboratory students who collected blood specimens in the field, supervised fieldwork and laboratory validation process, reviewed data analysis, and reviewed final report.

Mahmood N: developed proposal conceptualization, reviewed methodology step by step, followed technical progress of proposal and fieldwork though expert meetings, guided all administrative steps from proposal development to writing the final report, and participated in search for funding of the project.

Musani A: WR in Yemen, led approval of the study by WHO.

Baoom NM: Minister of Public Health and Population (Yemen) during planning and implementation of the study, led approval of the study by the Ministry of Public Health and Population.

Al-Waleedi A: proposal conceptualization, obtained official approval, supervised fieldwork, and reviewed final report.

Boliva E: proposal conceptualization, followed all technical aspects of proposal and fieldwork and formal data analysis, reviewed first draft, and secured funding of the project.

Naiene JD: proposal conceptualization, followed all technical aspects of proposal and fieldwork and formal data analysis, secured all laboratory logistics for fieldwork, and reviewed first draft of report.
Aly A: reviewed proposal, designed questionnaire, reviewed formal data analysis including maintenance of research data (software code) for data interpretation for initial use and subsequent re-use, and wrote first and final drafts of report.

Barakat A: prepared laboratory methodology, followed all steps of laboratory activities in the field and laboratory validation process, reviewed data analysis, and reviewed final report.

Crestani R, Senga M: participated actively in proposal conceptualization, followed all technical steps of fieldwork, reviewed first draft of data analysis, and reviewed first draft of final report.

Al-Sakkaf Kh AZ, Shaef A: trained data collectors, supervised fieldwork, and reviewed final report.

Thabit N: trained data collectors, supervised fieldwork, and secured all field and laboratory logistics.

Murshed A, Omara S: trained data collectors, supervised fieldwork, designed digital questionnaire, followed dashboards, finalized formal analysis, and maintained software for fieldwork and laboratory work.

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