Seroprevalence of anti–SARS-CoV-2 antibodies in women attending antenatal care in eastern Ethiopia

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Abstract

Information on the cumulative incidence of SARS-CoV-2 in East Africa is scarce. We conducted serosurveillance of anti–SARS-CoV-2 antibodies among pregnant women attending their first antenatal care visit in three health facilities in eastern Ethiopia.

We collected data using questionnaire and a blood sample from 3,312 pregnant women between April 1, 2020 and March 31, 2021 at health facilities in Haramaya, Aweday and Harar. We selected 1,447 blood samples at random and assayed these for anti-SARS-CoV-2 antibodies at Hararghe Health Research laboratory using WANTAI® SARS-CoV-2 Rapid Test for total immunoglobulin. Temporal trends in seroprevalence were analysed with a $\chi^2$ test for trend and multivariable binomial regression.

Among 1,447 sera tested, 83 were positive for anti–SARS-CoV-2 antibodies giving a crude seroprevalence of 5.7% (95% CI 4.6%, 7.0%). Of 160 samples tested in April-May, 2020, none was seropositive; the first seropositive sample was identified in June and seroprevalence rose steadily thereafter ($\chi^2$ test for trend, $p=0.003$) reaching a peak of 11.8% in February, 2021. In the multivariable model, seroprevalence was approximately 3% higher in first-trimester mothers compared to later presentations, and rose by 0.75% (95% CI 0.31%, 1.20%) per month of calendar time.

This clinical convenience sample illustrates the dynamic of the SARS-CoV-2 epidemic in young adults in eastern Ethiopia; infection was rare before June 2020 but it spread in a linear fashion thereafter, rather than following intermittent waves, and reached 10% by the beginning of 2021. After one year of surveillance, most pregnant mothers remained susceptible.
Keywords: COVID-19, COVID-19 seroprevalence, COVID-19 among pregnant women, COVID-19 in Ethiopia
Introduction

In Ethiopia, the first case of COVID-19 was reported on 13 March 2020. By the end of March 2021 there were 206,589 reports of COVID-19 infection and 2,865 coronavirus-related deaths. In a country with an estimated population, in 2019, of 112 million this represents a cumulative incidence of SARS-CoV-2 infection of only 0.2% after a full year of transmission. Many cases of COVID-19 present with mild symptoms and, in Ethiopia, three quarters of PCR-positive cases have no symptoms [1,2]. Access to PCR testing in Ethiopia is also sparse. Monitoring the epidemic by detecting symptomatic cases is, therefore, highly insensitive. In these circumstances, seroprevalence of anti-SARS-CoV-2 antibodies can provide a more accurate estimator of cumulative incidence. Undertaking community serosampling during the pandemic is difficult when travel and household access are constrained by control measures. Expectant mothers, however, are likely to continue to seek health services throughout the pandemic and they can be used as a continuously-available proxy population to estimate the cumulative incidence among young adults [3-5]. In addition, serological surveillance is simple to implement at ANC clinic visits because anti-SARS-CoV-2 antibodies can be assayed in the residual blood volumes of routine samples collected for clinical screening for anemia and maternal infectious diseases.

Planning and provision of health care during a major epidemic like COVID-19 pose substantial logistical and clinical challenges. Information on the shape of the epidemic curve is critical to inform public health responses. The dynamics of seroprevalence reflect the epidemic curve and can provide an estimate of the effective reproduction number. Seroprevalence also indicates the likelihood of approaching transmission control through population immunity. This study aimed
to assess the trend in seroprevalence of anti-SARS-CoV-2 antibodies throughout the first year of the epidemic by assaying anti-SARS-CoV-2 antibodies among pregnant women attending antenatal clinic at three different health facilities in the area around Harar, eastern Ethiopia.

Results

Demographic characteristics of the study participants

Between April 1, 2020 and March 31, 2021 there were 3,313 first visits to the antenatal clinics; 1,532 (46.24%) at Hiwot Fana Hospital, 1,781 (53.75%) at Aweday Health Centre and Haramaya Hospital. At these, we interviewed and collected blood samples from 3,312 women. We tested a random sample of 1,447 blood specimens (Table 1); 752 (52%) were from Haramaya District (Aweday Health Centre and Haramaya Hospital) and 695 (48%) were from Hiwot Fana Hospital; 984 (68%) were urban residents (Table 1).

Table 1. Characteristics of 1,447 pregnant mothers attending their first antenatal care at the two study areas between April 2020 and March 2021 and sampled at random for the study.

| Characteristics | Haramaya District | Hiwot Fana Hospital | Total |
|-----------------|-------------------|---------------------|-------|
|                 | n     | %     | n     | %     | n     | %     |
| Age in years    |       |       |       |       |       |       |
| 14-19           | 98    | 13.0  | 74    | 13.5  | 192   | 13.3  |
| 20-24           | 341   | 45.4  | 268   | 35.6  | 588   | 40.7  |
| 25-29           | 181   | 24.1  | 219   | 31.6  | 400   | 27.7  |
| 30-34           | 110   | 14.6  | 100   | 14.4  | 210   | 14.5  |
| >=35            | 22    | 2.9   | 35    | 4.9   | 56    | 3.9   |
| Residence       |       |       |       |       |       |       |
| Urban           | 577   | 76.8  | 407   | 58.7  | 984   | 68.1  |
| Rural           | 174   | 23.2  | 287   | 41.4  | 461   | 31.9  |
Among the population sample tested, the mean (SD) age was 23.9 (4.7) years and ages ranged from 15 to 45 years. The mean (SD) number of children per mother was 1.5 (1.8). The median (IQR) gestational age at the first antenatal visit was 20 (13-28) weeks. Only 51 (3.5%) had COVID-19 symptoms at the time of sampling and 8 (<1%) had a history of comorbidity, given as chronic liver, renal, cardiovascular or ‘other’ disease. Respiratory diseases, chronic neurological disease, diabetes mellitus, and cancer were not reported by any participant (Table 2).

Table 2. Seroprevalence of Anti-SARS-CoV-2 antibodies by participant characteristics.

| Characteristics | Tested | Sero-positive | Sero-prevalence | \(\chi^2\) test |
|-----------------|--------|---------------|-----------------|-----------------|

Notes:
* chronic liver, renal, cardiovascular or ‘other’ disease
† at least one of cough, fever, headache or difficulty breathing
### Age in years

| Age in years | N   | n | %  | p  |
|--------------|-----|---|----|----|
| 14-19        | 192 | 7 | 3.7| 0.19 |
| 20-24        | 588 | 42| 7.1|     |
| 25-29        | 400 | 24| 6.0|     |
| 30-34        | 210 | 9 | 4.3|     |
| >=35         | 56  | 1 | 1.8|     |

### ANC clinic

| ANC clinic                  | N   | n | %  | p  |
|-----------------------------|-----|---|----|----|
| Hiwot Fana Hospital         | 695 | 35| 5.0| 0.26|
| Haramaya Hospital           | 19  | 0 | 0.0|     |
| Aweday Health Centre        | 733 | 48| 6.6|     |

### Residence

| Residence | N   | n | %  | p  |
|-----------|-----|---|----|----|
| Urban     | 984 | 57| 5.8| 0.91|
| Rural     | 461 | 26| 5.6|     |

### Number of children

| None   | 1,439 | 81 | 5.6 | 0.019 |
| 1-5    | 778   | 45 | 5.5 |       |
| 6-10   | 55    | 6  | 9.8 |       |

### Trimester of visit

| Trimester | N   | n | %  | p  |
|-----------|-----|---|----|----|
| First     | 366 | 31| 8.5| 0.034|
| Second    | 658 | 32| 4.9|     |
| Third     | 423 | 20| 4.8|     |

### Comorbidities

| Comorbidities | N   | n | %  | p  |
|---------------|-----|---|----|----|
| None          | 1,439 | 81| 5.6 |     |
| At least one* | 8    | 2 | 25.0| 0.019|

### COVID symptoms†

| COVID symptoms† | N   | n | %  | p  |
|-----------------|-----|---|----|----|
| No              | 1,389 | 82| 5.9 | 0.24|
| Yes             | 51   | 1 | 2.0|     |

* chronic liver, renal, cardiovascular or ‘other’ disease
† at least one of cough, fever, headache or difficulty breathing

## Seroprevalence of SARS-CoV-2 antibodies

Of 1,447 samples tested, 83 (5.7%, 95% CI 4.6, 7.0%) were positive for anti-SARS-CoV-2 antibodies. The first seropositive sample was identified on June 11, 2020, and seroprevalence
rose progressively thereafter, with the exception of March 2021, where it dropped sharply ($\chi^2$ for trend for the whole year, $p=0.003$; Figure 1).

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**Figure 1**: Temporal trend of seroprevalence of anti–SARS-CoV-2 antibodies among pregnant women presenting for first antenatal care in 3 ANC facilities, eastern Ethiopia, between April 1, 2020 and March 31, 2021

Seroprevalence also varied significantly by trimester of pregnancy and co-morbidity but not by clinic, residence or COVID-19 symptoms (Table 2). Given the linear growth in seroprevalence (Figure 1) and better model fit based on Bayesian information criterion, we modelled prevalence
associations as risk differences rather than risk ratios. In a multivariable binomial regression model, the prevalence difference was -3.2% (95% CI -6.7, -0.4%) and -3.0% (95% CI -6.8, -0.8%) among women in their second and third trimesters, respectively, compared with those in the first trimester and the prevalence difference was 0.75% (95% CI 0.31, 1.20%) per month of calendar time.

Discussion

The study provides a simple description of the dynamic of SARS-CoV-2 epidemic in an area where reliable data are extremely rare. In a population of attendees at ante-natal clinics in three sites in eastern Ethiopia, antibodies against SARS-CoV-2 first appeared in June 2020 and seroprevalence rose steadily month on month reaching approximately 10% at the beginning of 2021. Although the point estimate for March 2021 is substantially lower, the data as a whole evince a strong linear trend and this single estimate is most likely to have deviated from the general direction by chance. If these results are reliable, they indicate that the epidemic is progressing here at a considerably lower rate than in other settings in East Africa and that the greater majority of the population remains uninfected, suggesting that the epidemic is still at an early stage.

The principal limitations of the study are the potential generalisability of the population under surveillance and the validity of the serological assay employed. Pregnant women have been used as an indicator population in prior pandemics, including HIV [6], but also for SARS-CoV-2, both in high-income settings [4,5,7-11] and low- and middle-income settings, including in neighbouring Kenya [3]. The principal advantage of sampling pregnant women is that they
remain one of the few patient groups for whom health services cannot be postponed until after
the pandemic has passed. They are permitted and encouraged to attend even in the face of social
and movement restrictions, and so provide a consistent and reliable sampling group. The
principal limitation of this group is their restriction on age and sex, however, in most settings,
including other East African countries, seroprevalence does not vary significantly by sex and the
cumulative incidence in women is likely to represent the infection history of both sexes [12].
Similarly, in most settings young adults are the group most likely to be infected by SARS-CoV-2
and so the seroprevalence estimates here are likely to represent the highest risk in the whole
population; other age groups, particularly children and the elderly, are likely to have lower
seroprevalence [13,14].

The World Health Organization has deprecated the use of rapid tests for SARS-CoV-2 antibodies
for individual diagnosis but recognises their potential value in research [15]. WHO has also
recommended and endorsed quantitative analysis of IgG antibodies using ELISA and has
distributed the WANTAI ELISA kit to countries undertaking serosurveillance. Reliance on
ELISA, however, limits the range of settings in which serosurveillance can be undertaken and
lateral flow tests have been successfully employed for recurrent community-based nationwide
surveys in the UK [16]. When seroprevalence is low, as at the beginning of our study, an assay
with imperfect specificity may detect more false positives than true positives. The specificity of
the WANTAI rapid test has been estimated by the manufacturer at 98.8%. We assayed 80
samples each month in April and May 2020 without observing a single positive test, suggesting
that the specificity is indeed very high. Even if the positive results identified in June included
false positives, the progressive rise in seropositivity with time is most unlikely to be influenced
materially by a small fraction of false positive results.
The assay sensitivity may also be imperfect in detecting prior infection because the assay was originally calibrated against sera from symptomatic cases, who generally have higher antibody levels than asymptomatic individuals [17], and because pregnant women who were infected several months ago may have experienced waning of antibody levels and seroreversion [18-22]. In general, seroreversion is less problematic in assays that measure total immunoglobulin and in those that target spike antigens, compared to nucleocapsid antigens [20,23], so problem of waning in this study is unlikely to be substantial. Furthermore, if sensitivity is unlikely to decline over time, imperfect sensitivity would not affect the shape of the rising seroprevalence line, though it would underestimate the gradient. If, as estimated in one validation study, the WANTAI rapid test has a sensitivity of only 89% [24], adjustment for test-performance characteristics would elevate our reported seroprevalence results by a factor of 1.12.

The results are in contrast to most other settings studied which record a sharp take-off in seroprevalence once transmission begins, often rising quickly to high levels. In Kenya for example, women attending an ANC clinic in Kilifi had seroprevalence of 0%, 2% and 11% in consecutive months September-November 2021; those attending ANC clinic in Nairobi had a seroprevalence of 50% in August 2020 [3]. The pattern illustrated in eastern Ethiopia is more indicative of a gradually spreading epidemic curve suggesting an effective reproduction number much closer to 1.

In Juba, South Sudan, seroprevalence was 22% in a household survey in August-September 2020 [25]; in Kenya, a national estimate for seroprevalence, based on testing blood transfusion donors, was 4.3% in May 2020 [12] and 9.1% two months later [26]. Health Care Workers in Nairobi, Kenya, had a seroprevalence of 44% in August 2020; those in two rural hospitals had
seroprevalence of 12-13% in November 2020 [27]. Finally, in Addis Ababa seroprevalence, estimated in May 2020, was 3.0% [28]. Although all these studies used different laboratory assays and varied statistical adjustments, collectively, they suggest that transmission in eastern Ethiopia began later than in much of the rest of the region, including the state capital, and has progressed more slowly.

In summary if seroprevalence is a reliable indicator of cumulative incidence, SARS-CoV-2 infection is spreading slowly but steadily in eastern Ethiopia. This contrasts sharply with the recurrent waves of PCR-positive infections apparent in the national surveillance system. One year after the start of the epidemic approximately 10% of women attending ante-natal clinics are seropositive implying that the COVID-19 epidemic is still at an early stage in eastern Ethiopia.

Material and methods

Study area and period

The surveillance was conducted between April 1, 2020 and March 31, 2021 at Aweday Health Centre and Haramaya District Hospital, both in Haramaya District, and in Hiwot Fana Specialized Referral University Hospital in Harar. Hiwot Fana is the largest referral and teaching hospital in eastern Ethiopia and receives tertiary referrals from Harari region, East Oromia, Somali region, and Dire Dawa City. It is one of the ten regional centres designated by the Federal Ministry of Health to manage the COVID-19 epidemic. Haramaya Hospital was rapidly designated a COVID-19 treatment facility and women seeking ANC services were therefore referred to Aweday Health Centre after April 16, 2020.
Study design, population and sample size

At the end of March 2020, we integrated Health facility-based surveillance into the routine clinical care of pregnant women at Hiwot Fana Hospital, Aweday Health Centre and Haramaya Hospital. The study population comprised 3306 pregnant women attending their first antenatal care in these three facilities during the surveillance period. A total 78 women were excluded because they were not willing to provide blood sample. Routine antenatal care includes serological screening for HIV, syphilis, and toxoplasma infection during pregnancy undertaken in two blood samples; the first blood sample is taken at 16 weeks' gestation or at the first ANC visit, if later.

Socio-demographic data and information on pregnancy, clinical symptoms of COVID-19 and co-morbidities was collected by trained nurses. COVID-19 symptoms were defined as at least one of cough, fever, headache or difficulty breathing. Data quality and completeness were checked daily.

Laboratory analyses

For the anti–SARS-CoV-2 antibodies test, residual blood samples from the routine ANC tests were transferred to a test-tube containing clot activator by trained medical laboratory technologists working in each health facility. The blood samples were allowed to clot and serum was separated by centrifugation at 3000 RPM for 10 minutes. Serum samples were stored at 2-8°C at each site and transported in cool boxes to Hararghe Health Research Laboratory where they were stored at -80°C.
Samples were tested using WANTAI® SARS-CoV-2 Ab Rapid Test. The test is a lateral flow assay in a cassette format designed for the qualitative detection of total antibodies to SARS-CoV-2 in human serum. The receptor-binding domain of the SARS-CoV-2 spike protein is bound at the Test Zone (T) and antibodies are bound at the Control Zone (C) of the cassette. The test has a sensitivity of 100% and specificity of 98.8% under validation performed by the manufacturer; independent validation of the test found a sensitivity of 89% [24]. All the stored serum samples, tests reagents and cassettes were brought to room temperature (15-30°C) thirty minutes before performing the test; 10μl of serum specimen and two drops of diluent buffer were added into the specimen window. Results were read and interpreted as reactive/positive (Red line on C and T) or non-reactive/negative (Red line on C) after 15-20 minutes [24,29]. Serum samples taken ≥14 days after a positive PCR test from COVID-19 infected individuals were used as quality control.

**Statistical analysis**

We used STATA version 16.0 for statistical analysis. We estimated unadjusted seroprevalence of SARS-CoV-2 IgG antibody with a 95% confidence interval (CI). We did not make adjustment for the test performance characteristics because the manufacturer’s validation assay, found very high sensitivity and specificity. We examined the univariate association between individual characteristics and seropositivity using $\chi^2$ and multivariable associations using binomial regression. The trend in seropositivity with time was tested with a $\chi^2$ test for trend and in the multivariable model.
Ethical consideration

The study was confined to residual clinical blood sample testing and anonymized questionnaire data. It was conducted as part of a public health surveillance, with the approval of the director of each of the three health facilities and the data were made available to relevant bodies including the Regional Health Bureau (Harari and Oromia) and the Ethiopian Public Health Institute (EPHI). Ethical clearance was secured from Institutional Health Research Ethical Review Committee of the College of Health and Medical Sciences, Haramaya University, Ethiopia.

Data availability

Data is available in the following link and can be requested using the form in the link.

https://dataverse.harvard.edu/dataset.xhtml?persistentId=doi:10.7910/DVN/XIWCXN.

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Author contribution

NA and JAGS lead overall Surveillance. NA, JAGS, ZT and LDR develop concept of the Surveillance, analyzed the data and wrote the manuscript. ZT provided microbiome data analysis, and interpretation. NA, JAGS, ZT, LDR, LM, JO and YD reviewed the manuscript and give critical feedback. All authors approved the submission of the manuscript to the journal.
Competing of interest

The authors declare no competing interests.
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