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

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3 1 **Seroprevalence of anti-SARS-CoV-2 antibodies in women attending antenatal**
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6 2 **care in eastern Ethiopia**
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29 Abstract

30 **Objective:** We conducted serosurveillance of anti-SARS-CoV-2 antibodies among pregnant
31 women attending their first antenatal care.

32 **Setting:** The surveillance was set in one referral hospital in Harar, one district hospital and one
33 health centre located in Haramaya district in rural eastern Ethiopia.

34 **Participants:** We collected questionnaire data and a blood sample from 3,312 pregnant women
35 between April 1, 2020 and March 31, 2021. We selected 1,447 blood samples at random and
36 assayed these for anti-SARS-CoV-2 antibodies at Hararghe Health Research laboratory using
37 WANTAI® SARS-CoV-2 Rapid Test for total immunoglobulin.

38 **Outcome:** We assayed for anti-SARS-CoV-2 antibodies and temporal trends in seroprevalence
39 were analysed with a χ^2 test for trend and multivariable binomial regression.

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

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

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2
3 51 **Keywords:** COVID 19, COVID-19 seroprevalence, COVID-19 among pregnant women, COVID-
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5 52 19 in Ethiopia
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8 53 **Key summaries:**

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10 **What is already known about this subject?**

- 11 54 • Information on the cumulative incidence of SARS-CoV-2 in is scarce and little is known
12 about.
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15 55 **What are the new findings?"**

- 16 • The crude seroprevalence anti-SARS-CoV-2 antibodies among first time ANC
17 attendants was 5.7%.
18 • The first seropositive sample was identified in June 2020 and seroprevalence rose
19 56 steadily thereafter reached a peak of 11.8% in February, 2021.
20 • Seroprevalence was approximately 3% higher in first-trimester mothers compared to later
21 presentations, and rose by 0.75% per month of calendar time.
22 57

23
24 **How might it impact on clinical practice in the foreseeable future?**

- 25 • The disease spreads among asymptomatic community members unnoticed.
26 58 • A healthy looking could be source of infection for susceptible individuals. This will
27 eventually create pressure on health facilities to treat symptomatic cases.
28 • The effect of COVID infection on birth outcomes needs to be investigated.
29 59

30
31 **Strengths and limitations**

32 **Strength**

- 33 60 • Selecting healthy looking first time ANC attendants to represent the general population
34 • Instituting a surveillance in both tertiary and primary health facilities located in urban
35 and rural areas represents the urban and rural community.
36

37 **Limitation**

- 38 61 • Its focus on women visited the health care facilities and missing several not attending
39 health care.
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65 Introduction

66 In Ethiopia, the first case of COVID-19 was reported on 13 March 2020. By the end of March
67 2021 there were 206,589 reports of COVID-19 infection and 2,865 coronavirus-related deaths. In
68 a country with an estimated population, in 2019, of 112 million this represents a cumulative
69 incidence of SARS-CoV-2 infection of only 0.2% after a full year of transmission. Many cases of
70 COVID-19 present with mild symptoms and, in Ethiopia, three quarters of PCR-positive cases
71 have no symptoms [1,2]. Access to PCR testing in Ethiopia is also sparse. Monitoring the epidemic
72 by detecting symptomatic cases is, therefore, highly insensitive. In these circumstances,
73 seroprevalence of anti-SARS-CoV-2 antibodies can provide a more accurate estimator of
74 cumulative incidence. Undertaking community serosampling during the pandemic is difficult
75 when travel and household access are constrained by control measures. Expectant mothers,
76 however, are likely to continue to seek health services throughout the pandemic and they can be
77 used as a continuously-available proxy population to estimate the cumulative incidence among
78 young adults [3-5]. In addition, serological surveillance is simple to implement at ANC clinic visits
79 because anti-SARS-CoV-2 antibodies can be assayed in the residual blood volumes of routine
80 samples collected for clinical screening for anemia and maternal infectious diseases.

81 Planning and provision of health care during a major epidemic like COVID-19 pose substantial
82 logistical and clinical challenges. Information on the shape of the epidemic curve is critical to
83 inform public health responses. The dynamics of seroprevalence reflect the epidemic curve and
84 can provide an estimate of the effective reproduction number. Seroprevalence also indicates the
85 likelihood of approaching transmission control through population immunity. This study aimed to
86 assess the trend in seroprevalence of anti-SARS-CoV-2 antibodies throughout the first year of the

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3 87 epidemic by assaying anti-SARS-CoV-2 antibodies among pregnant women attending ante-natal
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5 88 clinic at three different health facilities in the area around Harar, eastern Ethiopia.
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10 89 **Material and methods**

11 12 13 90 **Study area and period**

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16 91 The surveillance was conducted between April 1, 2020 and March 31, 2021 at Awoday Health
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18 92 Centre and Haramaya District Hospital, both in Haramaya District, and in Hiwot Fana Specialized
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20 93 Referral University Hospital in Harar. Hiwot Fana is the largest referral and teaching hospital in
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22 94 eastern Ethiopia and receives tertiary referrals from Harari region, East Oromia, Somali region,
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24
25 95 and Dire Dawa City. It is one of the ten regional centres designated by the Federal Ministry of
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27 96 Health to manage the COVID-19 epidemic. Haramaya Hospital was rapidly designated a COVID-
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29 97 19 treatment facility and women seeking ANC services were therefore referred to Awoday Health
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31
32 98 Centre after April 16, 2020.
33

34 99 **Study design, population and sample size**

35
36 100 At the end of March 2020, we integrated Health facility-based surveillance into the routine clinical
37
38 101 care of pregnant women at Hiwot Fana Hospital, Awoday Health Centre and Haramaya Hospital.
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41 102 The study population comprised 3306 pregnant women attending their first antenatal care in these
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43 103 three facilities during the surveillance period. A total 78 women were excluded because they were
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45 104 not willing to provide blood sample. Routine antenatal care includes serological screening for HIV,
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48 105 syphilis, and toxoplasma infection during pregnancy undertaken in two blood samples; the first
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50 106 blood sample is taken at 16 weeks' gestation or at the first ANC visit, if later.
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3 107 Socio-demographic data and information on pregnancy, clinical symptoms of COVID-19 and co-
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5 108 morbidities was collected by trained nurses. COVID-19 symptoms were defined as at least one of
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8 109 cough, fever, headache or difficulty breathing. Data quality and completeness were checked daily.
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11 111 **Laboratory analyses**

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14 112 For the anti-SARS-CoV-2 antibodies test, residual blood samples from the routine ANC tests were
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17 113 transferred to a test-tube containing clot activator by trained medical laboratory technologists
18
19 114 working in each health facility. The blood samples were allowed to clot and serum was separated
20
21 115 by centrifugation at 3000 RPM for 10 minutes. Serum samples were stored at 2-8°C at each site
22
23 116 and transported in cool boxes to Hararghe Health Research Laboratory where they were stored at
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26 117 -80°C.

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28 118 Samples were tested using WANTAI® SARS-CoV-2 Ab Rapid Test. The test is a lateral flow
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31 119 assay in a cassette format designed for the qualitative detection of total antibodies to SARS-CoV-2
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33 120 in human serum. The receptor-binding domain of the SARS-CoV-2 spike protein is bound at the
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35 121 Test Zone (T) and antibodies are bound at the Control Zone (C) of the cassette. The test has a
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37 122 sensitivity of 100% and specificity of 98.8% under validation performed by the manufacturer;
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39 123 independent validation of the test found a sensitivity of 89% [6]. All the stored serum samples,
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41 124 tests reagents and cassettes were brought to room temperature (15-30°C) thirty minutes before
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43 125 performing the test; 10µl of serum specimen and two drops of diluent buffer were added into the
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45 126 specimen window. Results were read and interpreted as reactive/positive (Red line on C and T) or
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47 127 non-reactive/negative (Red line on C) after 15-20 minutes [6,7]. Serum samples taken ≥14 days
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49 128 after a positive PCR test from COVID-19 infected individuals were used as quality control.
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131 **Patient and Public Involvement statement**

132 In COVID pandemic, much attention have been given for COVID symptomatic cases, while
133 asymptomatic cases are either not addressed or neglected. In this study, it has been discussed with
134 public health authorities and community representatives in the study area in several occasions.
135 Women have much interaction in the community as well in the family. They represent the
136 community better than other person to learn the dynamics of a disease of such kind. For this reason,
137 a surveillance was set among pregnant women attending ANC in local health facilities. These
138 women come to health facilities for check-up, and investigators agreed that, first time ANC visitors
139 are best representatives.

140 As clients were visiting the antenatal clinic, nurses at the clinic explained the issue of COVID and
141 asked women if she would like to participate by giving blood sample for testing. Pregnant women
142 were encouraged to raise any unclear points and then referred to the laboratory for blood sample
143 collection as they do other tests.

144 Data from this surveillance is made available in public repository. Aggregated data has been shared
145 to authorities in districts and health facilities to plan for actions against the problem. Pregnant
146 women attending ANC were given information on the disease nationally, regionally and locally,
147 and they were also given health education on COVID prevention.

148

149 **Statistical analysis**

150 We used STATA version 16.0 for statistical analysis. We estimated unadjusted seroprevalence of
151 SARS-CoV-2 IgG antibody with a 95% confidence interval (CI). We did not make adjustment for
152 the test performance characteristics because the manufacturer's validation assay, found very high
153 sensitivity and specificity. We examined the univariate association between individual

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3 154 characteristics and seropositivity using χ^2 and multivariable associations using binomial
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6 155 regression. The trend in seropositivity with time was tested with a χ^2 test for trend and in the
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8 156 multivariable model.
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11 12 13 158 **Ethical consideration**

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15 159 The study was confined to residual clinical blood sample testing and anonymized questionnaire
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17 160 data. It was conducted as part of a public health surveillance, with the approval of the director of
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19 161 each of the three health facilities and the data were made available to relevant bodies including the
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21 162 Regional Health Bureau (Harari and Oromia) and the Ethiopian Public Health Institute (EPHI).
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23 163 Ethical clearance was secured from Institutional Health Research Ethical Review Committee of
24
25 164 the College of Health and Medical Sciences, Haramaya University, Ethiopia with clearance
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27 165 number 123/2021.
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33 166 **Results**

34 35 36 37 167 **Demographic characteristics of the study participants**

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39 168 Between April 1, 2020 and March 31, 2021 there were 3,313 first visits to the antenatal clinics;
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41 169 1,532 (46.24%) at Hiwot Fana Hospital, 1781 (53.75%) at Awoday Health Centre and Haramaya
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43 170 Hospital. At these, we interviewed and collected blood samples from 3,312 women. We tested a
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45 171 random sample of 1,447 blood specimens (Table 1); 752 (52%) were from Haramaya District
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47 172 (Awoday Health Centre and Haramaya Hospital) and 695 (48%) were from Hiwot Fana Hospital;
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49 173 984 (68%) were urban residents.
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53 174 Among the population sample tested, the mean (SD) age was 23.9 (4.7) years and ages ranged
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55 175 from 15 to 45 years. The mean (SD) number of children per mother was 1.5 (1.8). The median
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176 (IQR) gestational age at the first antenatal visit was 20 (13-28) weeks. Only 51 (3.5%) had COVID-
177 19 symptoms at the time of sampling and 8 (<1%) had a history of comorbidity, given as chronic
178 liver, renal, cardiovascular or 'other' disease. Respiratory diseases, chronic neurological disease,
179 diabetes mellitus, and cancer were not reported by any participant.

180 **Seroprevalence of SARS-CoV-2 antibodies**

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

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

193 **Discussion**

194 The study provides a simple description of the dynamic of SARS-CoV-2 epidemic in an area where
195 reliable data are extremely rare. In a population of attendees at ante-natal clinics in three sites in
196 eastern Ethiopia, antibodies against SARS-CoV-2 first appeared in June 2020 and seroprevalence
197 rose steadily month on month reaching approximately 10% at the beginning of 2021. Although the
198 point estimate for March 2021 is substantially lower, the data as a whole evince a strong linear

199 trend and this single estimate is most likely to have deviated from the general direction by chance.

200 If these results are reliable, they indicate that the epidemic is progressing here at a considerably
201 lower rate than in other settings in East Africa and that the greater majority of the population
202 remains uninfected, suggesting that the epidemic is still at an early stage.

203 The principal limitations of the study are the potential generalisability of the population under
204 surveillance and the validity of the serological assay employed. Pregnant women have been used
205 as an indicator population in prior pandemics, including HIV [8], but also for SARS-CoV-2, both
206 in high-income settings [4,5,9-13] and low- and middle-income settings, including in neighbouring
207 Kenya [3]. The principal advantage of sampling pregnant women is that they remain one of the
208 few patient groups for whom health services cannot be postponed until after the pandemic has
209 passed. They are permitted and encouraged to attend even in the face of social and movement
210 restrictions, and so provide a consistent and reliable sampling group. The principal limitation of
211 this group is their restriction on age and sex, however, in most settings, including other East
212 African countries, seroprevalence does not vary significantly by sex and the cumulative incidence
213 in women is likely to represent the infection history of both sexes [14]. Similarly, in most settings
214 young adults are the group most likely to be infected by SARS-CoV-2 and so the seroprevalence
215 estimates here are likely to represent the highest risk in the whole population; other age groups,
216 particularly children and the elderly, are likely to have lower seroprevalence [15,16].

217 The World Health Organization has deprecated the use of rapid tests for SARS-CoV-2 antibodies
218 for individual diagnosis but recognises their potential value in research [17]. WHO has also
219 recommended and endorsed quantitative analysis of IgG antibodies using ELISA and has
220 distributed the WANTAI ELISA kit to countries undertaking serosurveillance. Reliance on
221 ELISA, however, limits the range of settings in which serosurveillance can be undertaken and

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3 222 lateral flow tests have been successfully employed for recurrent community-based nationwide
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5 223 surveys in the UK [18]. When seroprevalence is low, as at the beginning of our study, an assay
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7 224 with imperfect specificity may detect more false positives than true positives. The specificity of
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9 225 the WANTAI rapid test has been estimated by the manufacturer at 98.8%. We assayed 80 samples
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11 226 each month in April and May 2020 without observing a single positive test, suggesting that the
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13 227 specificity is indeed very high. Even if the positive results identified in June included false
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15 228 positives, the progressive rise in seropositivity with time is most unlikely to be influenced
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17 229 materially by a small fraction of false positive results.
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21 230 The assay sensitivity may also be imperfect in detecting prior infection because the assay was
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23 231 originally calibrated against sera from symptomatic cases, who generally have higher antibody
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25 232 levels than asymptomatic individuals [19], and because pregnant women who were infected
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27 233 several months ago may have experienced waning of antibody levels and seroreversion [20-24].
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29 234 In general, seroreversion is less problematic in assays that measure total immunoglobulin and in
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31 235 those that target spike antigens, compared to nucleocapsid antigens [22,25], so problem of waning
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33 236 in this study is unlikely to be substantial. Furthermore, if sensitivity is unlikely to decline over
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35 237 time, imperfect sensitivity would not affect the shape of the rising seroprevalence line, though it
36
37 238 would underestimate the gradient. If, as estimated in one validation study, the WANTAI rapid test
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39 239 has a sensitivity of only 89% [6], adjustment for test-performance characteristics would elevate
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41 240 our reported seroprevalence results by a factor of 1.12.
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47 241 The results are in contrast to most other settings studied which record a sharp take-off in
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49 242 seroprevalence once transmission begins, often rising quickly to high levels. In Kenya for example,
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51 243 women attending an ANC clinic in Kilifi had seroprevalence of 0%, 2% and 11% in consecutive
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53 244 months September-November 2021; those attending ANC clinic in Nairobi had a seroprevalence
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3 245 of 50% in August 2020 [3]. The pattern illustrated in eastern Ethiopia is more indicative of a
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5 246 gradually spreading epidemic curve suggesting an effective reproduction number much closer to
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10 248 In Juba, South Sudan, seroprevalence was 22% in a household survey in August-September 2020
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12 249 [26]; in Kenya, a national estimate for seroprevalence, based on testing blood transfusion donors,
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14 250 was 4.3% in May 2020 [14] and 9.1% two months later [27]. Health Care Workers in Nairobi,
15
16 251 Kenya, had a seroprevalence of 44% in August 2020; those in two rural hospitals had
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18 252 seroprevalence of 12-13% in November 2020 [28]. Finally, in Addis Ababa seroprevalence,
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20 253 estimated in May 2020, was 3.0% [29]. Although all these studies used different laboratory assays
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22 254 and varied statistical adjustments, collectively, they suggest that transmission in eastern Ethiopia
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24 255 began later than in much of the rest of the region, including the state capital, and has progressed
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26 256 more slowly.

30 31 **Conclusion**

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34 258 In summary if seroprevalence is a reliable indicator of cumulative incidence, SARS-CoV-2
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36 259 infection is spreading slowly but steadily in eastern Ethiopia. This contrasts sharply with the
37
38 260 recurrent waves of PCR-positive infections apparent in the national surveillance system. One year
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40 261 after the start of the epidemic approximately 10% of women attending ante-natal clinics are
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42 262 seropositive implying that the COVID-19 epidemic is still at an early stage in eastern Ethiopia.

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339 **Author contribution**

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24 340 NA and JAGS lead overall Surveillance. NA, JAGS, ZT and LDR develop concept of the
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26 341 Surveillance, analyzed the data and wrote the manuscript. ZT provided microbiome data analysis,
27
28 342 and interpretation. NA, JAGS, ZT, LDR, LM, JO and YD reviewed the manuscript and give critical
29
30 343 feedback. All authors approved the submission of the manuscript to the journal.
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35 344 **Competing of interest**

36
37 345 The authors declare no competing interests.
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47
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5 351 **Data availability**

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8 352 Data is available in the following link and can be requested using the form in the link.

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10 353 <https://dataverse.harvard.edu/dataset.xhtml?persistentId=doi:10.7910/DVN/XIWCXN>.

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16
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18
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363 Figure 1: Temporal trend of seroprevalence of anti-SARS-CoV-2 antibodies among pregnant
364 women presenting for first antenatal care in 3 ANC facilities, eastern Ethiopia, between April
365 1, 2020 and March 31, 2021

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For peer review only

371 Table 1. Characteristics of 1,447 pregnant mothers attending their first antenatal care at the two
 372 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
Number of children						
None	298	40.0	254	36.8	552	38.4
1-5	422	56.6	401	58.1	823	57.3
6-10	26	3.5	35	5.1	61	4.3
Trimester of visit						
First	223	29.7	143	20.6	366	25.3
Second	416	55.3	242	34.8	658	45.5
Third	113	15.0	310	44.6	423	29.2
Comorbidities						
None	750	99.7	689	99.1	1,439	99.5
At least one*	2	0.3	6	0.9	8	0.6
COVID symptoms†						
No	721	96.4	668	96.5	1,389	96.5
Yes	27	3.6	24	3.5	51	3.5

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374 * chronic liver, renal, cardiovascular or 'other' disease

375 † at least one of cough, fever, headache or difficulty breathing

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

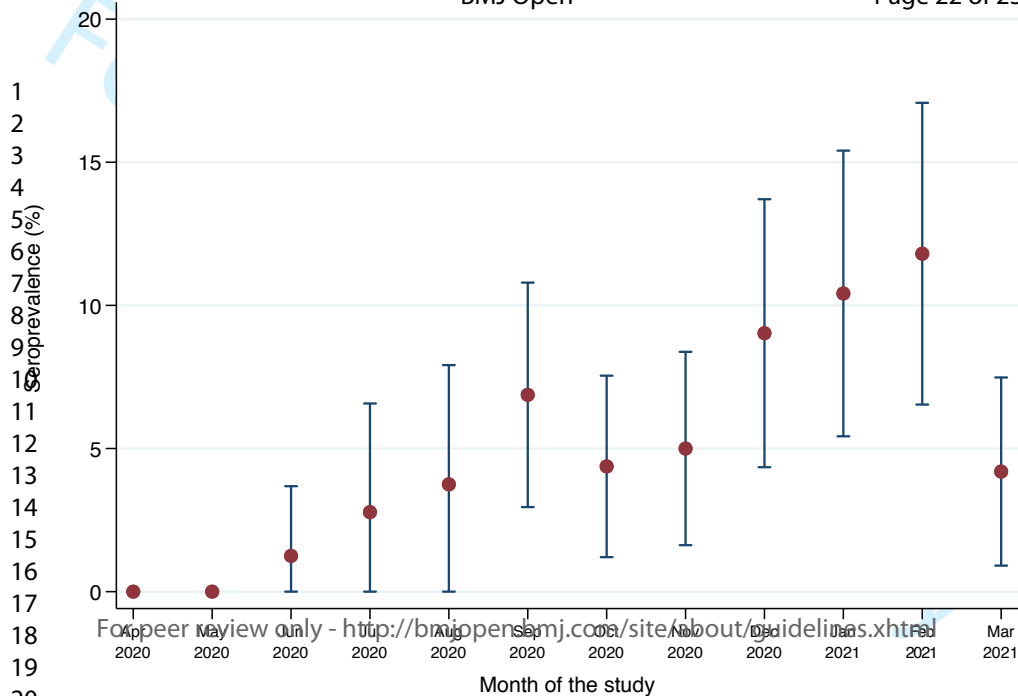
Characteristics	Tested N	Sero- positive n	Sero- prevalence %	χ^2 test
Age in years				
14-19	192	7	3.7	p=0.19
20-24	588	42	7.1	
25-29	400	24	6.0	
30-34	210	9	4.3	
>=35	56	1	1.8	
ANC clinic				
Hiwot Fana Hospital	695	35	5.0	p=0.26
Haramaya Hospital	19	0	0.0	
Awoday Health Centre	733	48	6.6	
Residence				
Urban	984	57	5.8	p=0.91
Rural	461	26	5.6	
Number of children				
None	520	32	5.8	P=0.370
1-5	778	45	5.5	
6-10	55	6	9.8	
Trimester of visit				
First	366	31	8.5	p=0.034
Second	658	32	4.9	
Third	423	20	4.8	
Comorbidities				
None	1,439	81	5.6	p=0.019
At least one*	8	2	25.0	
COVID symptoms†				
No	1,389	82	5.9	p=0.24
Yes	51	1	2.0	

378

379 * chronic liver, renal, cardiovascular or 'other' disease

380 † at least one of cough, fever, headache or difficulty breathing

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

STROBE Statement—Checklist of items that should be included in reports of *cross-sectional studies*

	Item No	Recommendation	Page No
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	1
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4-5
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants	5-6
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6
Data sources/measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7
Bias	9	Describe any efforts to address potential sources of bias	7
Study size	10	Explain how the study size was arrived at	5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6-7
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	7
		(b) Describe any methods used to examine subgroups and interactions	7
		(c) Explain how missing data were addressed	7
		(d) If applicable, describe analytical methods taking account of sampling strategy	7
		(e) Describe any sensitivity analyses	7
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8
		(b) Give reasons for non-participation at each stage	8
		(c) Consider use of a flow diagram	-
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8-10
		(b) Indicate number of participants with missing data for each variable of interest	8
Outcome data	15*	Report numbers of outcome events or summary measures	8-9

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

Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	8-9
		(b) Report category boundaries when continuous variables were categorized	9
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	-
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	9
Discussion			
Key results	18	Summarise key results with reference to study objectives	9-10
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	10
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	10-12
Generalisability	21	Discuss the generalisability (external validity) of the study results	11-12
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	16

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Seroprevalence of anti-SARS-CoV-2 antibodies in women attending antenatal care in eastern Ethiopia: a facility-based surveillance

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5 **1 Seroprevalence of anti-SARS-CoV-2 antibodies in women attending antenatal**
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7 **2 care in eastern Ethiopia: a facility-based surveillance**
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10 3 Nega Assefa^{1, 2, *}, Lemma Demissie Regassa¹, Zelalem Teklemariam¹, Joseph Oundo², Lola
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25 Abstract 245

26 Main text 2489
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29 Abstract

30 **Objective:** We conducted serosurveillance of anti-SARS-CoV-2 antibodies among pregnant
31 women attending their first antenatal care.

32 **Setting:** The surveillance was set in one referral hospital in Harar, one district hospital and one
33 health centre located in Haramaya district in rural eastern Ethiopia.

34 **Participants:** We collected questionnaire data and a blood sample from 3,312 pregnant women
35 between April 1, 2020 and March 31, 2021. We selected 1,447 blood samples at random and
36 assayed these for anti-SARS-CoV-2 antibodies at Hararghe Health Research laboratory using
37 WANTAI® SARS-CoV-2 Rapid Test for total immunoglobulin.

38 **Outcome:** We assayed for anti-SARS-CoV-2 antibodies and temporal trends in seroprevalence
39 were analysed with a Chi square test for trend and multivariable binomial regression.

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

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

1
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3 51 **Keywords:** COVID 19, COVID-19 seroprevalence, COVID-19 among pregnant women, COVID-
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5 52 19 in Ethiopia
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10 54 **Strengths and limitations**
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15 56 • The surveillance was initiated quickly at the start of the pandemic and was pursued with
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17 57 consistent methods over a full calendar year
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19 58 • Pregnant women are consistently available for surveillance throughout movement restrictions
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21 59 providing a practical and valid survey of seroprevalence trends
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24 60 • Results from pregnant women may not be fully representative of older or younger women,
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26 61 nor of men at any age.
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29 62 • SARS-CoV-2 antibodies were assayed using a lateral flow device which, though convenient,
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31 63 has inferior performance characteristics to ELISA
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65 Introduction

66 In Ethiopia, the first case of COVID-19 was reported on 13 March 2020. By the end of March
67 2021, there were 206,589 reports of COVID-19 infection and 2,865 coronavirus-related deaths. In
68 a country with an estimated population, in 2019, of 112 million this represents a cumulative
69 incidence of SARS-CoV-2 infection of only 0.2% after a full year of transmission. Many cases of
70 COVID-19 present with mild symptoms and, in Ethiopia, three quarters of PCR-positive cases
71 have no symptoms [1 2](#). Access to PCR testing in Ethiopia is also sparse. Monitoring the epidemic
72 by detecting symptomatic cases is, therefore, highly insensitive. In these circumstances,
73 seroprevalence of anti-SARS-CoV-2 antibodies can provide a more accurate estimator of
74 cumulative incidence. Undertaking community sero-sampling during the pandemic is difficult
75 when travel and household access are constrained by control measures. Expectant mothers,
76 however, are likely to continue to seek health services throughout the pandemic and they can be
77 used as a continuously available proxy population to estimate the cumulative incidence among
78 young adults [3-5](#). In addition, serological surveillance is simple to implement at Antenatal Clinic
79 (ANC) visits because anti-SARS-CoV-2 antibodies can be assayed in the residual blood volumes
80 of routine samples collected for clinical screening for anaemia and maternal infectious diseases.

81 Planning and provision of health care during a major epidemic like COVID-19 pose substantial
82 logistical and clinical challenges. Information on the shape of the epidemic curve is critical to
83 inform public health responses. The dynamics of seroprevalence reflect the epidemic curve and
84 can provide an estimate of the effective reproduction number. Seroprevalence also indicates the
85 likelihood of approaching transmission control through population immunity. This study aimed to
86 assess the trend in seroprevalence of anti-SARS-CoV-2 antibodies throughout the first year of the

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3 87 epidemic by assaying anti-SARS-CoV-2 antibodies among pregnant women attending ANC at
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5 88 three different health facilities in the area around Harar, eastern Ethiopia.
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10 89 **Material and methods**

11 12 13 90 **Study area and period**

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15 91 The surveillance was conducted between April 1, 2020 and March 31, 2021 at Awoday Health
16
17 92 Centre and Haramaya District Hospital, both in Haramaya District, and in Hiwot Fana Specialized
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19 93 Referral University Hospital in Harar. Hiwot Fana is the largest referral and teaching hospital in
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21 94 eastern Ethiopia and receives tertiary referrals from Harari region, East Oromia, Somali region,
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23 95 and Dire Dawa City. It is one of the ten regional centres designated by the Federal Ministry of
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25 96 Health to manage the COVID-19 epidemic. Haramaya Hospital was rapidly designated a COVID-
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27 97 19 treatment facility and women seeking ANC services were therefore referred to Awoday Health
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29 98 Centre after April 16, 2020. Ethiopia began to roll out COVID-19 vaccine in the first quarter of
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31 99 2021; however, no doses were given in the study area during the period this analysis covers.
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38 100 **Study design, population and sample size**

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40 101 At the end of March 2020, we integrated Health facility-based surveillance into the routine clinical
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42 102 care of pregnant women at Hiwot Fana Hospital, Awoday Health Centre and Haramaya Hospital.
43
44 103 The study population comprised 3306 pregnant women attending their first antenatal care in these
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46 104 three facilities during the surveillance period. Because we had fewer test kits available than there
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48 105 were samples available from the clinic, we selected a random sample for analysis stratified on
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50 106 month. Initially, we decided to select 144 samples per month; as the seroprevalence was very low
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52 107 in the first three months we reset the sample size to 80 per month from July onwards and increased
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3 108 it to 160 per month in December once the seroprevalence reached 5%. In October, the number of
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5 109 samples collected was lower than the desired sample size and we therefore tested all of the samples
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8 110 (See Supplementary table).
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12 111 A total of 78 women were excluded because they were not willing to provide a blood sample.
13
14 112 Routine antenatal care includes serological screening for HIV, syphilis, and toxoplasma infection
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16 113 during pregnancy undertaken in two blood samples; the first blood sample is taken at 16 weeks'
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18 114 gestation or at the first ANC visit, if later.
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23 115 Socio-demographic data and information on pregnancy, clinical symptoms of COVID-19 and co-
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25 116 morbidities was collected by trained nurses. COVID-19 symptoms were defined as at least one of
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27 117 cough, fever, headache or difficulty breathing. Data quality and completeness were checked daily.
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31 32 118 **Laboratory analyses** 33

34 119 For the anti-SARS-CoV-2 antibodies test, residual blood samples from the routine ANC tests were
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36 120 transferred to a test-tube containing clot activator by trained medical laboratory technologists
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38 121 working in each health facility. The blood samples were allowed to clot and serum was separated
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40 122 by centrifugation at 3000 RPM for 10 minutes. Serum samples were stored at 2-8°C at each site
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42 123 and transported in cool boxes to Hararghe Health Research Laboratory where they were stored at
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44 124 -80°C.
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50 125 Samples were tested using WANTAI® SARS-CoV-2 Ab Rapid Test. The test is a lateral flow
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52 126 assay in a cassette format designed for the qualitative detection of total antibodies to SARS-CoV-2
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54 127 in human serum. The receptor-binding domain of the SARS-CoV-2 spike protein is bound at the
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3 128 Test Zone (T) and antibodies are bound at the Control Zone (C) of the cassette. The test has a
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5 129 sensitivity of 100% and specificity of 98.8% under validation performed by the manufacturer [6](#);
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8 130 independent validation of the test found a sensitivity of 89% [7](#). All the stored serum samples, tests
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10 131 reagents and cassettes were brought to room temperature (15-30°C) thirty minutes before
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12 132 performing the test and checked for defects. Then, a 10µl of serum specimen and two drops of
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14 133 diluent buffer were added into the specimen window. Results were read and interpreted as
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16 134 reactive/positive (Red line on C and T) or non-reactive/negative (Red line on C) after 15-20
17
18 135 minutes according to manufacturer's instruction [67](#). Serum samples taken ≥ 14 days after a positive
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20 136 PCR test from COVID-19 infected individuals were used as positive control. Samples were tested
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22 137 in batches of 50-60 by a single operator. Assays without a valid reaction on the control line were
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24 138 rejected and the assay repeated on a new kit.
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30 139 **Patient and Public Involvement statement**

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32 140 Because the surveillance was set up urgently at the beginning of the pandemic we were not able to
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34 141 involve participants or the public in the design or set-up and because it was designed as an
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36 142 anonymous surveillance we were not able to provide individual feedback of the results to the
37
38 143 participants. We have provided feedback of these high-level results through our existing
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40 144 community engagement exercises, including local radio programmes, meetings with local leaders
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42 145 and communication through health workers for onward dissemination.
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48 146 **Statistical analysis**

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50 147 We used STATA version 16.0 for statistical management and analysis. We selected a random
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52 148 sample of participants each month using the runiform function. We estimated unadjusted
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54 149 seroprevalence of SARS-CoV-2 IgG antibody with a 95% confidence interval (CI). We did not
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3 150 make adjustment for the test performance characteristics because the manufacturer's validation
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5 151 assay, found very high sensitivity and specificity. We examined the univariate association between
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7 152 individual characteristics and seropositivity using Chi-square test and multivariable associations
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9
10 153 using binomial regression. The trend in seropositivity with time was tested with a Chi-square test
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12 154 for trend and in the multivariable model. Data used in the analysis is available at
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15 155 dataverse.harvard.edu [8](#).

19 156 **Ethical consideration**

21 157 The surveillance was confined to residual clinical blood sample testing and anonymized data were
22
23 158 collected using checklists to extract data from ANC cards. Bar codes, representing an anonymous
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26 159 unique identity number, were used to link extracted clinical and demographic data with test sample
27
28 160 results. The exercise was conducted as part of a public health surveillance, with the approval of
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30
31 161 the directors of each of the three health facilities, and the results were made available to health
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33 162 facilities, the Regional Health Bureau (Harari and Oromia) and the Ethiopian Public Health
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35 163 Institute (EPHI). All ANC attendees were informed that the clinic was participating in an
36
37 164 anonymous surveillance and mothers were made aware that the residual volumes of their blood
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40 165 samples would be made available to the surveillance laboratory. Written individual informed
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42 166 consent was not obtained. The surveillance exercise was approved by the Institutional Health
43
44 167 Research Ethical Review Committee of the College of Health and Medical Sciences, Haramaya
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47 168 University, Ethiopia with clearance number 123/2021.

169 **Results**

170 **Demographic characteristics of the study participants**

171 Between April 1, 2020 and March 31, 2021 there were 3,313 first visits to the antenatal clinics;
172 1,532 (46.24%) at Hiwot Fana Hospital, 1781 (53.75%) at Awoday Health Centre and Haramaya
173 Hospital. At these, we interviewed and collected blood samples from 3,312 women. We tested a
174 random sample of 1,447 blood specimens (Table 1); 752 (52%) were from Haramaya District
175 (Awoday Health Centre and Haramaya Hospital) and 695 (48%) were from Hiwot Fana Hospital;
176 984 (68%) were urban residents.

177 Among the population sample tested, the mean (SD) age was 23.9 (4.7) years and ages ranged
178 from 15 to 45 years. The mean (SD) number of children per mother was 1.5 (1.8). The median
179 (IQR) gestational age at the first antenatal visit was 20 (13-28) weeks. Only 51 (3.5%) had COVID-
180 19 symptoms at the time of sampling and 8 (<1%) had a history of comorbidity, given as chronic
181 liver, renal, cardiovascular or 'other' disease. Respiratory diseases, chronic neurological disease,
182 diabetes mellitus, and cancer were not reported by any participant.

183 **Seroprevalence of SARS-CoV-2 antibodies**

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

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2
3 188 Seroprevalence also varied significantly by trimester of pregnancy and co-morbidity but not by
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5 189 clinic, residence or COVID-19 symptoms (Table 2). Given the linear growth in seroprevalence
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7 190 (Figure 1) and better model fit based on Bayesian information criterion, we modelled prevalence
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9 191 associations as risk differences rather than risk ratios. In a multivariable binomial regression
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11 192 model, the prevalence difference was -3.2% (95% CI -6.7, -0.4%) and -3.0% (95% CI -6.8,-0.8%)
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13 193 among women in their second and third trimesters, respectively, compared with those in the first
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15 194 trimester and the prevalence difference was 0.75% (95% CI 0.31, 1.20%) per month of calendar
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17 195 time.
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24 196 **Discussion**

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26 197 The study provides a simple description of the dynamic of SARS-CoV-2 epidemic in an area where
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28 198 reliable data are extremely rare. In a population of attendees at antenatal clinics in three sites in
29
30 199 eastern Ethiopia, antibodies against SARS-CoV-2 first appeared in June 2020 and seroprevalence
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32 200 rose steadily month on month reaching approximately 10% at the beginning of 2021. Although the
33
34 201 point estimate for March 2021 is substantially lower, the data as a whole evince a strong linear
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36 202 trend and this single estimate is most likely to have deviated from the general direction by chance.
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38 203 If these results are reliable, they indicate that the epidemic is progressing here at a considerably
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40 204 lower rate than in other settings in East Africa and that the greater majority of the population
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42 205 remains uninfected, suggesting that the epidemic is still at an early stage.
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49 206 The principal limitations of the study are the potential generalisability of the population under
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51 207 surveillance and the validity of the serological assay employed. Pregnant women have been used
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53 208 as an indicator population in prior pandemics, including HIV [9](#), but also for SARS-CoV-2, both in
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3 209 high-income settings [4 5 10-14](#) and low- and middle-income settings, including in neighbouring
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5 210 Kenya [3](#). The principal advantage of sampling pregnant women is that they remain one of the few
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8 211 patient groups for whom health services cannot be postponed until after the pandemic has passed.
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10 212 They are permitted and encouraged to attend even in the face of social and movement restrictions,
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12 213 and so provide a consistent and reliable sampling group. The principal limitation of this group is
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14 214 their restriction on age and sex, however, in most settings, including other East African countries,
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16 215 seroprevalence does not vary significantly by sex and the cumulative incidence in women is likely
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18
19 216 to represent the infection history of both sexes [15-20](#). Similarly, in most settings young adults are
20
21 217 the group most likely to be infected by SARS-CoV-2 and so the seroprevalence estimates here are
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23 218 likely to represent the highest risk in the whole population; other age groups, particularly children
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25 219 and the elderly, are likely to have lower seroprevalence [17 21](#).

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30 220 The World Health Organization has deprecated the use of rapid tests for SARS-CoV-2 antibodies
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32 221 for individual diagnosis but recognises their potential value in research [22](#). WHO has also
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34 222 recommended and endorsed quantitative analysis of IgG antibodies using ELISA and has
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36 223 distributed the WANTAI ELISA kit to countries undertaking serosurveillance. Reliance on
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38 224 ELISA, however, limits the range of settings in which serosurveillance can be undertaken and
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40 225 lateral flow tests have been successfully employed for recurrent community-based nationwide
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42 226 surveys in the UK [23](#). When seroprevalence is low, as at the beginning of our study, an assay with
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44 227 imperfect specificity may detect more false positives than true positives. The specificity of the
45
46 228 WANTAI rapid test has been estimated by the manufacturer at 98.8%. We assayed 80 samples
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48 229 each month in April and May 2020 without observing a single positive test, suggesting that the
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50 230 specificity is indeed very high. Even if the positive results identified in June included false
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3 231 positives, the progressive rise in seropositivity with time is most unlikely to be influenced
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5 232 materially by a small fraction of false positive results.
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10 233 The assay sensitivity may also be imperfect in detecting prior infection because the assay was
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12 234 originally calibrated against sera from symptomatic cases, who generally have higher antibody
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14 235 levels than asymptomatic individuals [24](#), and because pregnant women who were infected several
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16 236 months ago may have experienced waning of antibody levels and seroreversion [25-29](#). In general,
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18 237 seroreversion is less problematic in assays that measure total immunoglobulin and in those that
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20 238 target spike antigens, compared to nucleocapsid antigens [27 30](#), so problem of waning in this study
21
22 239 is unlikely to be substantial. Furthermore, if sensitivity is unlikely to decline over time, imperfect
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24 240 sensitivity would not affect the shape of the rising seroprevalence line, though it would
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26 241 underestimate the gradient. If, as estimated in one validation study, the WANTAI rapid test has a
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28 242 sensitivity of only 89% [7](#), adjustment for test-performance characteristics would elevate our
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30 243 reported seroprevalence results by a factor of 1.12.
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37 244 The results are in contrast to most other settings studied which record a sharp take-off in
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39 245 seroprevalence once transmission begins, often rising quickly to high levels. In Kenya for example,
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41 246 women attending an ANC clinic in Kilifi had seroprevalence of 0%, 2% and 11% in consecutive
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43 247 months September-November 2021; those attending ANC clinic in Nairobi had a seroprevalence
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45 248 of 50% in August 2020 [3](#). The pattern illustrated in eastern Ethiopia is more indicative of a
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47 249 gradually spreading epidemic curve suggesting an effective reproduction number much closer to
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3 251 In Juba, South Sudan, seroprevalence was 22% in a household survey in August-September 2020
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5 252 [20](#); in Kenya, a national estimate for seroprevalence, based on testing blood transfusion donors,
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7 253 was 4.3% in May 2020 [18](#) and 9.1% two months later [15](#). Health Care Workers in Nairobi, Kenya,
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9 254 had a seroprevalence of 44% in August 2020; those in two rural hospitals had seroprevalence of
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11 255 12-13% in November 2020 [15](#). Finally, in Addis Ababa seroprevalence, estimated in May 2020,
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13 256 was 3.0% [31](#). Although all these studies used different laboratory assays and varied statistical
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15 257 adjustments, collectively, they suggest that transmission in eastern Ethiopia began later than in
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17 258 much of the rest of the region, including the state capital, and has progressed more slowly.
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24 259 **Conclusion**

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26 260 In summary, if seroprevalence is a reliable indicator of cumulative incidence, SARS-CoV-2
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28 261 infection is spreading slowly but steadily in eastern Ethiopia. This contrasts sharply with the
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30 262 recurrent waves of PCR-positive infections apparent in the national surveillance system. One year
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32 263 after the start of the epidemic, approximately 10% of women attending antenatal clinics are
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34 264 seropositive implying that the COVID-19 epidemic is still at an early stage in eastern Ethiopia.
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43 266 **[Dataset]** [8] Assefa N, Demissie L, Teklemariam Z, Oundo J, Madrid L, Dessie Y, et al. *Data*
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45 267 *from: Seroprevalence of anti-SARS-CoV-2 antibodies in women attending antenatal care in*
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47 268 *eastern Ethiopia: V1 ed: Harvard Dataverse; June 9, 2021. doi:10.7910/DVN/XIWCXN*
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372 **Author contribution**

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22 373 NA and JAGS lead overall Surveillance. NA, JAGS, ZT and LDR develop concept of the
23 374 Surveillance, analysed the data and wrote the manuscript. ZT provided microbiome data analysis,
24 375 and interpretation. NA, JAGS, ZT, LDR, LM, JO and YD reviewed the manuscript and give critical
25 376 feedback. All authors approved the submission of the manuscript to the journal.
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377 **Competing of interest**

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34 378 The authors declare no competing interests.
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41
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5 384 **Data availability**
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8 385 Data is available in the following link and can be requested using the form in the link.
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10 386 <https://dataverse.harvard.edu/dataset.xhtml?persistentId=doi:10.7910/DVN/XIWCXN>.
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19 389 and pregnant women for voluntarily participating in the study.
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3 391 **Figure titles/legends**
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7 392 Figure 1: Temporal trend of seroprevalence of anti-SARS-CoV-2 antibodies among pregnant
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10 393 women presenting for first antenatal care in 3 ANC facilities, eastern Ethiopia, between April
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12 394 1, 2020 and March 31, 2021
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For peer review only

396 Table 1. Characteristics of 1,447 pregnant mothers attending their first antenatal care at the two
 397 study clinics 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
Number of children						
None	298	40.0	254	36.8	552	38.4
1-5	422	56.6	401	58.1	823	57.3
6-10	26	3.5	35	5.1	61	4.3
Trimester of visit						
First	223	29.7	143	20.6	366	25.3
Second	416	55.3	242	34.8	658	45.5
Third	113	15.0	310	44.6	423	29.2
Comorbidities						
None	750	99.7	689	99.1	1,439	99.5
At least one*	2	0.3	6	0.9	8	0.6
COVID symptoms†						
No	721	96.4	668	96.5	1,389	96.5
Yes	27	3.6	24	3.5	51	3.5

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399 * chronic liver, renal, cardiovascular or 'other' disease

400 † at least one of cough, fever, headache or difficulty breathing

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

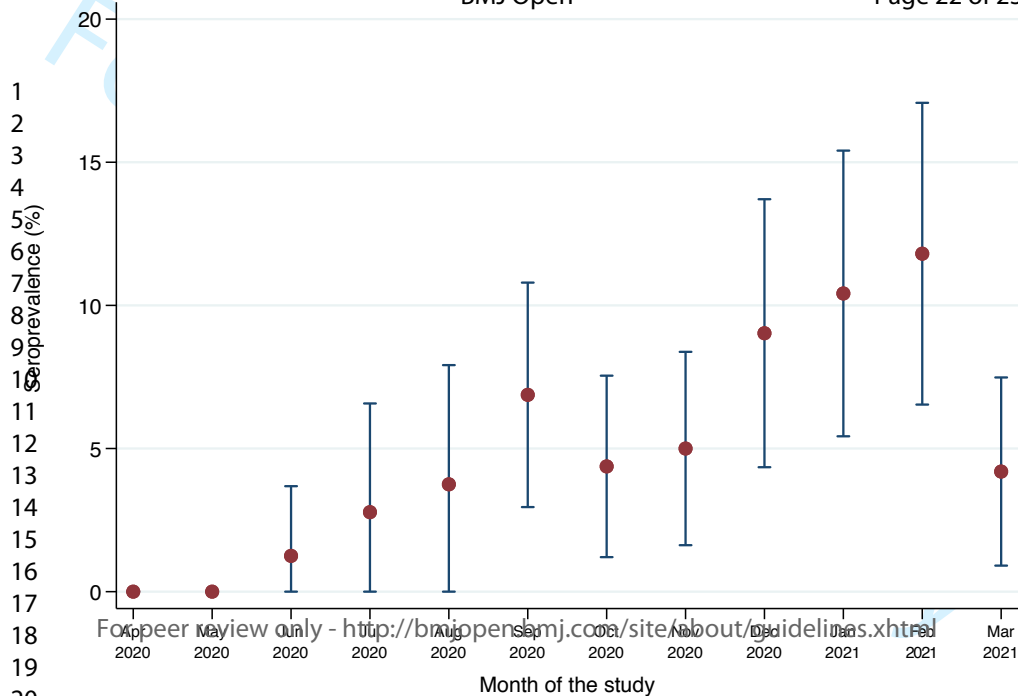
Characteristics	Tested N	Sero-positive n	Sero-prevalence %	Chi-square test p value
Age in years				
14-19	192	7	3.7	0.19
20-24	588	42	7.1	
25-29	400	24	6.0	
30-34	210	9	4.3	
>=35	56	1	1.8	
ANC clinic				
Hiwot Fana Hospital	695	35	5.0	0.260
Haramaya Hospital	19	0	0.0	
Awoday Health Centre	733	48	6.6	
Residence				
Urban	984	57	5.8	0.910
Rural	461	26	5.6	
Number of children				
None	520	32	5.8	0.370
1-5	778	45	5.5	
6-10	55	6	9.8	
Trimester of visit				
First	366	31	8.5	0.034
Second	658	32	4.9	
Third	423	20	4.8	
Comorbidities				
None	1,439	81	5.6	0.019
At least one*	8	2	25.0	
COVID symptoms†				
No	1,389	82	5.9	0.240
Yes	51	1	2.0	

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404 * chronic liver, renal, cardiovascular or 'other' disease

405 † at least one of cough, fever, headache or difficulty breathing

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

STROBE Statement—Checklist of items that should be included in reports of *cross-sectional studies*

	Item No	Recommendation	Page No
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	1
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4-5
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants	5-6
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6
Data sources/measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7
Bias	9	Describe any efforts to address potential sources of bias	7
Study size	10	Explain how the study size was arrived at	5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6-7
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	7
		(b) Describe any methods used to examine subgroups and interactions	7
		(c) Explain how missing data were addressed	7
		(d) If applicable, describe analytical methods taking account of sampling strategy	7
		(e) Describe any sensitivity analyses	7
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8
		(b) Give reasons for non-participation at each stage	8
		(c) Consider use of a flow diagram	-
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8-10
		(b) Indicate number of participants with missing data for each variable of interest	8
Outcome data	15*	Report numbers of outcome events or summary measures	8-9

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

Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	8-9
		(b) Report category boundaries when continuous variables were categorized	9
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	-
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	9
Discussion			
Key results	18	Summarise key results with reference to study objectives	9-10
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	10
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	10-12
Generalisability	21	Discuss the generalisability (external validity) of the study results	11-12
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	16

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.