SARS-CoV-2 in rural Latin America. A population-based study in coastal Ecuador.

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ABSTRACT

Antibodies to SARS-CoV-2 were detected in 303/673 rural Ecuadorian adults (45%), 77% of whom had compatible clinical manifestations. Seropositivity was associated with the use of open latrines. Our findings support the fears of mass spread of SARS-CoV-2 in rural Latin America and cannot exclude a contributing role for fecal-oral transmission.

Key Words: SARS-CoV-2; COVID-19; Coronavirus-19; Population study; Rural setting; Ecuador.

The global SARS-CoV-2 pandemic has spread to rural areas of developing countries [1,2]. These people are likely to be especially vulnerable to this pandemic because of factors inherent to under-development [3]. However, there is little or nil evidence of the burden and profile of SARS-CoV-2 infections in remote rural settings.

The Atahualpa Project is a population-based prospective cohort studying individuals aged ≥40 years in rural Ecuador, starting on 2012 [4]. An abrupt increase of deaths in the village was noticed in April and May, 2020 (overall mortality rate: 21.6‰ population, almost three-quarters of it attributable to SARS-CoV-2, configuring a 266% of excess mortality), that coincided with the peak of this pandemic in Ecuador, suggesting an outbreak of SARS-CoV-2 infection. Taking the opportunity of the above-mentioned well-established cohort, we assessed the prevalence, distribution, and risk factors associated with SARS-CoV-2 seropositivity in middle-aged and older adults living in this closed population.

METHODS

Atahualpa is located in Coastal Ecuador. The village has electricity and almost all houses have piped water. A sizable proportion of houses do not have flushing toilet systems and still use open latrines. Inhabitants are homogeneous regarding race/ethnicity (Amerindian ancestry), lifestyles, and diet. Almost all men belong to the blue-collar class (artisan carpenters) and most women are homemakers. Atahualpa has a low index of migration and the adherence of the population to the Atahualpa Project has been high, which makes it an optimal setting for conducting population-based studies [5].

For the present study, field personnel (including medical doctors) visited all houses of Atahualpa Project participants. Demographic data and risk factors were updated during house visits. Individuals were examined to assess current or past (prior two months) clinical manifestations suggestive of COVID-19 disease, according to the recommendations of the World Health Organization [6]. Detection of SARS-CoV-2 IgM and IgG antibodies was performed using a lateral flow antibody test (BIOHIT Health Care Ltd., Cheshire, UK) in finger prick blood samples. The manufacturer reports 97.5% sensitivity with 99.5% specificity for IgM, and 97.5% sensitivity with 100% specificity for IgG detection of this kit. Results of those tests were independently reviewed by two readers, with excellent Kappa coefficients for interrater agreement (0.91). Discrepancies were resolved by consensus. The study followed the standards for reporting of observational studies in epidemiology (STROBE) guidelines [7], and the Independent Review Board of Universidad Espiritu Santo (IORG: 0010320; FWA: 00028878) approved the study protocol and informed consent forms.

Covariates were selected if they have been suggested to play a role in disease acquisition or spread, or in the development of clinical manifestations, and included age, gender, level of education, alcohol intake, number of individuals living in the house, number of bedrooms, having an open latrine, home confinement during the past two months, and cardiovascular health status, according to the American Heart Association criteria (smoking status, diet, physical activity, body mass index, blood pressure, fasting glucose, and total cholesterol blood levels) [8].

Data analyses were carried out by using STATA version 16 (College Station, TX, USA). In univariate analyses, continuous variables were compared by linear models and categorical variables by the x^2 or Fisher exact test as appropriate. Mixed logistic regression models, where subjects were clustered within blocks, and each block was considered a panel of random intercept, were fitted using seropositivity to SARS-CoV-2 antibodies as the dependent variable, adjusted for the above-mentioned covariates.

RESULTS

From 730 individuals aged ≥40 years that were actively enrolled in the Atahualpa Project as of May 31, 2019, 39 had either died (n=24) or emigrated (n=15) in the past 12 months. Thus, 691 were living in the village as of May 25, 2020, and from these, 18 declined participation in the current survey (coverage 97.4%).

The mean age of the 673 participants was 59.2±12.8 years, 381 (57%) were women, 354 (53%) had primary school education only, and 142 (21%) disclosed heavy alcohol intake. A poor cardiovascular health status was noticed in 453 (67%) participants, and the mean number of metrics in the poor range was 1.1±1 per person. A total of 238 (35%) individuals had been confined at home during the past two months. The 673 participants inhabited in a total of 411 houses, with a mean of 6.6±4.7 persons per house (including young adults and children), and these houses had a mean of 2.6±1.1 bedrooms. Open latrines were used by 137 (20%) individuals.

A total of 303 (45%) individuals were seropositive to SARS-CoV-2 antibodies (including 247 who were reactive to both IgM and IgG, nine to only IgM, and 47 to only IgG). Seropositive individuals were spread across the village (Figure 1). As IgM and IgG responses in SARS-CoV-2 develop with only a few days of difference, we defined seropositivity as a positive response to any of them. COVID-19-related clinical manifestations were recalled by 232 (77%) seropositive and 55 (15%) seronegative individuals. Twelve symptomatic seropositive patients required oxygen therapy, and other three needed artificial ventilator support. No patient died during the survey.

In univariate analyses, seropositive individuals presented more often COVID-19 related symptomatology, used more often open latrines than their seronegative counterparts, and fewer of them had been confined at home (Table 1). Multivariate analyses confirmed independent associations between seropositivity to SARS-CoV-2 antibodies and compatible symptomatology (OR: 24.3; 95% C.I.: 15.3 – 38.8; $p<0.001$) as well as use of open latrines (OR: 2.14; 95% C.I.: 1.23 – 1.72; $p=0.007$).

DISCUSSION

This study demonstrates a high (45%) seroprevalence to SARS-CoV-2, disseminated across the entire village, confirming fears of mass spread of the disease in rural populations of Latin America [9]. Despite almost nil migration into Atahualpa, many men travel to neighboring towns to trade the furniture they build as artisan carpenters. Atahualpa's scenario is typical of closed populations where inhabitants are immunologically naïve to a rapidly spreading pathogen. Rural populations of Latin America are additionally burdened by poor social determinants of health and inadequate access to medical care, and do not seem to be prepared for this pandemic at all [3].

Most available data about COVID-19-related predisposing factors derives from clinical series in health centers, with only few urban community surveys covering sampling proportions of the target population [10,11]. There are no similar community-wide studies reported from remote rural settings. In these settings, living conditions, risk factors and access to health care, are totally different than those in urban centers or in developed countries.

Factors associated with SARS-CoV-2 seropositivity in univariate analyses included the presence of COVID-19 related symptomatology and the use of open latrines. In contrast, confinement to home was inversely associated with seropositivity. A multivariate model confirmed the associations of seropositivity with clinical manifestations and the use of latrines. We cannot conclude that presence of asymptomatic seropositive patients points to true asymptomatic cases. This was a cross-sectional survey, and both recall bias and the occurrence of other infections, could have contributed to it. Also, higher rates of asymptomatic infections than in our study were reported in Veneto, Italy [10]. Likewise, a Swiss study found a seroprevalence of about 11% after five weeks of the first demonstrated case, with most individuals being asymptomatic [11].

On the basis of the demonstration of SARS-CoV-2 in feces [12], it has been suggested that the fecal-oral route may contribute to the transmission of this pathogen in rural areas of the developing world [3]. The present study provides indirect support to the above-mentioned hypothesis. Open latrines are often associated with inadequate handling of human feces, which may facilitate SARS-CoV-2 transmission. However, we cannot rule out the possibility that latrines are acting as a proxy for other unknown interacting variables. Further studies are needed to confirm the role of the fecal-oral route as a source of SARS-CoV-2 infection in rural settings.

Our study is the first to demonstrate the magnitude of the expansion of SARS-CoV-2 and the factors associated with seropositivity in a remote rural village. Another strength of this study include the high coverage and unbiased inclusion of long-term participants in the Atahualpa Project cohort, in whom several risk factors and conditions have been well characterized. This will provide grounds for the conduction of ambispective studies aimed to asses long-term consequences of COVID-19 infection. Nevertheless, the study has limitations. The study population was limited to individuals aged ≥40 years. As such, we missed the infection status of the younger villagers and how it could have influenced the overall seroprevalence. Individuals recently infected could be in the process of building their antibody response and would have tested negative. In addition, we cannot rule out a small degree of misclassification due to false positive or false negative results. The number of individuals with COVID-19-related clinical manifestations may be an overestimation due to poor specificity of the variable or recall bias.

CONCLUSION

This study demonstrate a high prevalence of seropositivity to SARS-CoV-2 in a closed rural population, as well as the independent association between seropositivity and COVID-19-related symptoms and the use of open latrines. Our findings confirm assumptions of mass dissemination of COVID-19 disease in immunological naïve rural settings and depict the initially devastating consequences of this infection.

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LEGEND FOR FIGURE

Figure 1 – Google map of Atahualpa (Google Earth, Google Inc., Mountain View, CA) showing the distribution of infected individuals (red dots) across the village. Overlapping dots correspond to persons living in the same house.

Table 1 – Characteristics of the study population according to serological status (univariate analysis).

	Total series (n=673)	**Seronegative (n=370)**	**Seropositive (n=303)**	***p* value**
Age, years (mean ± SD)	59·2 ± 12·8	59·7 ± 13	58·6 ± 12·6	0·244
Females, n (%)	381 (57)	209 (56)	172 (57)	0·942
Primary school education, n (%)	354 (53)	192 (52)	162 (53)	0·684
Alcohol intake ≥50 g/day, n (%)	142 (21)	73 (20)	69 (23)	0·336
Poor cardiovascular health status, n (%)	453 (67)	241 (65)	212 (70)	0·184
Poor health metrics per person (mean ± SD)	1·1 ± 1	1·15 ± 0·98	1·2 ± 1	0·122
Confined to home for 2 months, n (%)	238 (35)	143 (39)	95 (31)	0·049*
Previous or current symptoms of the disease, n (%)	287 (43)	54 (15)	233 (77)	<0·001*
Number of persons in the house (mean ± SD)	5·5 ± 3·3	5·4 ± 3·1	5·7 ± 3·4	0.327
Number of bedrooms in the house (mean ± SD)	2·6 ± 1·1	2·6 ± 1·1	2·5 ± 1·1	0·195
Use of open latrines, n (%)	137 (20)	63 (17)	74 (24)	0·018*

* Statistically significant result

Figure 1