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SARS-CoV-2 prevalence at eight urban health clinics in Nicaragua: possible implications for the COVID-19 pandemic

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ABSTRACT

Objective: To assess the prevalence of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) in selected health clinics in the three largest urban areas in Nicaragua, where data regarding coronavirus disease 2019 (COVID-19) testing, morbidity and mortality is severely limited.

Methods: In this cross-sectional study, participants were tested for SARS-CoV-2 RNA by loop-mediated isothermal amplification (LAMP), and were tested for antibodies using immunoassays. A questionnaire recorded subjects' COVID-19-associated symptoms and risk factors. Data were collected from 22 February to 19 March 2021, 1 year after the first confirmed cases of SARS-CoV-2 in Nicaragua. Study participants were enrolled while attending routine check-ups or seeking care unrelated to COVID-19. Study participation was random and voluntary. All patients were eligible to participate. Symptom history was not part of the eligibility criteria.

Results: The prevalence of current SARS-CoV-2 infection was high (14%, LAMP-positive/seronegative). Antibody testing showed higher overall seroprevalence (38%). Cough was the symptom most strongly associated with being LAMP-positive (odds ratio 3.57, 95% confidence interval 2.65–4.81). Loss of smell had the highest positive predictive value, and was significantly associated with being LAMP-positive.

Conclusion: The prevalence of current SARS-CoV-2 infection and seropositivity were fairly high. More than half of the sample population had evidence of current or past infection. Knowledge of this previously unknown elevated level of infection is crucial for healthcare providers and policy makers.

Introduction

Coronavirus disease 2019 (COVID-19) continues to present a major global healthcare challenge, putting a considerable strain on societies, particularly in small developing nations with limited financial resources. Successful efforts to curb the COVID-19 pandemic have included a variety of measures to reduce contagion, such as early and widespread testing, along with extensive contact tracing and quarantine. Population-based studies on the prevalence of infection at national and local levels have become essential tools for assessing the effectiveness of these containment strategies. However, disproportionately few studies have been conducted in the least developed countries.

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) was first detected in Nicaragua on 18 March 2020. Nevertheless, 1 year into the pandemic, little is known about the prevalence and characteristics of COVID-19 among the populace and its true health impact. Nicaragua´s response to COVID-19 deviates from the strategies of other Central American nations. It is the only country in the region to avoid tackling the spread of SARS-CoV-2 and the resulting COVID-19 head on, and lacks a well-defined, effective plan to reduce contagion. As a result, the virus has spread to a large proportion of the population, according to independent research and medical groups (Citizen Observatory, 2020). Real data on the degree of spread is non-existent. The Nicaraguan Government restricts testing for the virus to one government test centre in the nation's capital, and has not released complete infection statistics to the public or regional health organizations. Any statistics that do get published by the health ministry are suspect for their incongruous and greatly reduced levels of contagion and death compared with neighbouring countries, which have stringent containment strategies. The ratio of excess mortality to the officially reported COVID-19 death count has

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been estimated at 53 times (excess/official), which is the second highest in the world (Karlinsky and Kobak, 2021). The Pan American Health Organization has expressed frustration with the lack of complete and verifiable data on the pandemic in Nicaragua (Associated Press, 2020), as it is impractical to manage a crisis without knowing the extent of the problem.

To add to the limited existing knowledge in this area, this study assessed the prevalence of SARS-CoV-2 at eight urban hospitals and clinics in Nicaragua, including data on age, gender, symptoms and risk factors of study participants. These data are important for clinicians and public health workers, and to formulate policies for containing the spread of SARS-CoV-2. Additionally, these data are increasingly important for assessing Nicaragua´s COVID-19 response 1 year into the pandemic.

Methods

Study design and settings

This non-controlled, cross-sectional, non-interventional, observational study investigated the point prevalence of SARS-CoV-2 infection as determined by loop-mediated isothermal amplification (LAMP) of saliva samples and the presence of SARS-CoV-2 antibodies in whole blood.

A COVID-19 network was established to conduct this multi-centre research in Managua, León and Masaya, three of the most populated cities of Nicaragua representing 36% of the total population (PRONicaragua, 2020). This COVID-19 network is comprised of 22 physicians, including specialists in infectious diseases and paediatrics from eight health clinics.

From 22 February to 19 March 2021, study staff at participating health clinics recruited patients by inviting them to enrol in the study. On the day of specimen collection, an investigator in charge of recruiting volunteers invited patients attending routine check-ups or seeking care for issues unrelated to COVID-19 to participate in the study. Study participation on application was random and voluntary. All patients were eligible to participate. Individuals who expressed interest in participating were informed of the study´s purpose, how to fill out the questionnaire, and were asked to sign a form indicating informed consent. None of the study participants were asked about their possible exposure to the SARS-CoV-2 virus prior to recruitment. Symptom history was not part of the eligibility criteria. In the case of children and adolescents, their parents/guardians were invited to enrol their children in the study.

All participants, independent of symptom presentation, were tested for SARS-CoV-2 RNA through the LAMP detection method, and tested for the presence of SARS-CoV-2 antibodies through serological assays (Zhang et al., 2020a,b; Huete-Pérez et al., 2021a).

Survey tool

A questionnaire was employed to identify possible exposures or risk factors for infection, current symptoms, and factors associated with vulnerability to severe illness and complications (Table S1, see online supplementary material). These included age, sex, occupation, chronic medical conditions, and a history of close contact with confirmed cases. The questionnaire also gathered data on use of face masks, handwashing habits, and presence of a family member with COVID-19 symptoms at home. Physicians filled out the questionnaires while interviewing participants or children´s parents.

LAMP testing

Between 1 and 4 mL of saliva were self-collected by each study participant aged ≥5 years in flasks containing 2 mL of sample buffer for LAMP detection of SARS-CoV-2 (Rodino et al., 2020). LAMP has previously demonstrated sensitivity of 97.5% and specificity of 99.7% for detection of SARS-CoV-2 compared with reverse transcription polymerase chain reaction (RT-PCR) (Dao Thi et al., 2020; Yan et al., 2020). Experimental details of LAMP reactions have been described previously (Huete-Pérez et al., 2021a). After collection, specimens were kept on ice and transported immediately to the University of Central America for the detection of nucleic acids from SARS-CoV-2. Testing assays were performed on saliva samples without an RNA purification step within 1–2 h of collection. All LAMP reactions were performed according to the manufacturer's instructions using WarmStart Colorimetric LAMP 2X Master Mix (NEB, M1800L) (Zhang et al., 2020a,b). Briefly, the reaction mixtures (20 μL each) were prepared as follows: 10 μL of 2X Master Mix, 2 μL of 10X primer mix targeting viral genes N and E, 5 μL of nuclease-free water, and 3 μL of sample (NEB E2019 COVID LAMP kit). LAMP reactions were incubated at 65°C for 45 min. Photographs were taken, using mobile phone cameras, of testing tubes laid on white sheets of paper for visual colour-based detection of amplification reactions. Pink indicated a negative result and yellow indicated positive detection of SARS-CoV-2. Following the manufacturer's recommendations, control reactions were run without template (replaced with water) to ensure amplification specificity. When invalid/inconclusive results appeared due to insufficient sample volume or incorrect procedural techniques, testing was repeated. Results were communicated to physicians within 6 h, who in turn conveyed the results, along with appropriate medical advice, to the corresponding parents or legal guardians.

SARS-CoV-2 antibody detection

Blood was tested for the presence of SARS-CoV-2 immunoglobulin G (IgG) and IgM antibodies using the COVID-19 IgG/IgM Rapid Test (Safe-Care Bio-Tech, Hangzhou, China), which is a lateral flow immunoassay intended for qualitative detection and differentiation of IgM and IgG antibodies to SARS-CoV-2 in human venous whole blood, plasma or serum. The testing device uses standard colloidal gold antigen-conjugated particles as a tracer to detect human antibodies. The reported clinical sensitivity and specificity of the test are ≥91% and ≥96%, respectively. Tests were conducted as recommended by the manufacturer, using rigorous biosecurity and microbiological practices (Tang et al., 2020). The test requires approximately 20 μL of blood which is obtained from the finger using a single-use lancet. Blood was pipetted directly to the absorbent sample pad of the test device. Immediately after blood deposition, two drops of buffer, included in each kit, were added to the sample pad to start the flow of the sample up the lateral flow strip. Results of the antibody test were read 20 min following sample deposition by three independent laboratory technicians, and in the presence of weak bands, results were scored based on the majority reading.

For quality control and to validate the antibody detection protocol in the laboratory, 20 tests were performed on individuals who had been diagnosed by RT-PCR using nasopharyngeal swabs at the central government laboratory: eight were SARS-CoV-2-negative and 12 were SARS-CoV-2-positive. Eleven of the 12 SARS-CoV-2-positive individuals had positive antibody results with the rapid test.

Statistical analysis

Statistical analyses were carried out using R Version 3.6.3, a free software environment for statistical computing and graphics distributed under the Free Software Foundation's GNU General Public License (The R Foundation, www.r-project.org,) and STATA Version 17.0 (Stata Corp., College Station, TX, USA). Categorical variables were displayed as number and percentage. Positive results of SARS-CoV-2 by test method and participants' characteristics were reported. Tests of statistical significance were performed by calculation of Chi-squared statistics derived from 2×2 contingency tables on categorical data. Fisher's exact test was used as appropriate for small category sizes. Two-tailed distributions were assumed in derivation of *P*-values, with Bonferroni's correction

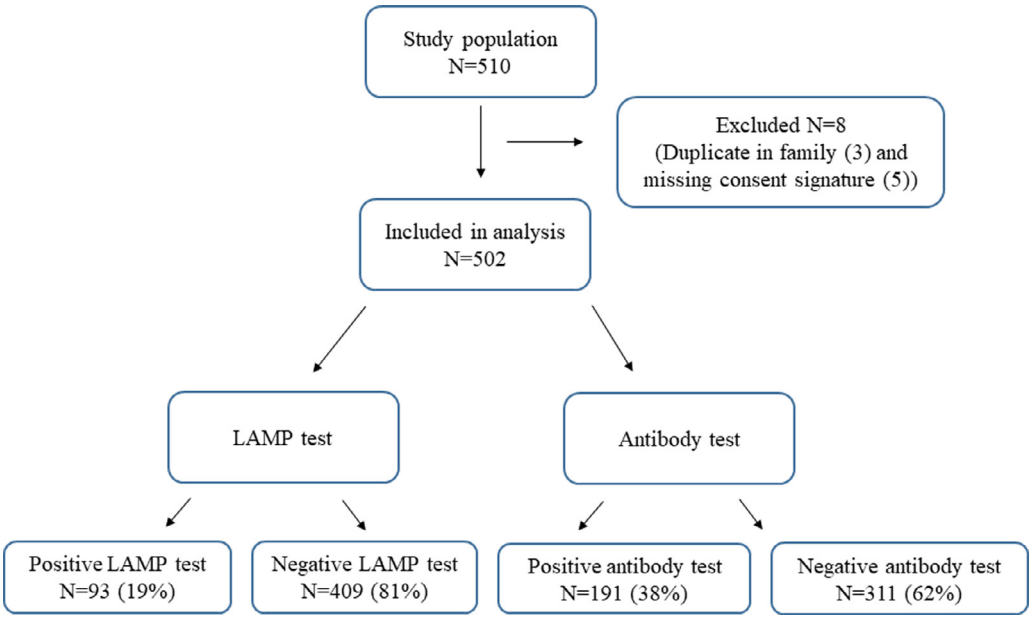

Figure 1. Study flow chart. LAMP, loop-mediated isothermal amplification.

Table 1
Demographic data of study participants.

Category	All ages	0–18 years	19–39 years	40–59 years	≥60 years
Number	502	78	208	156	60
Percentage	100	15.54	41.43	31.08	11.95
Sex (female/male)	274/228	38/40	112/96	93/63	31/29
Health clinic location					
Managua, *n* (%)	451 (89.84)	74 (94.87)	194 (93.27)	134 (85.90)	49 (81.67)
Leon and Masaya, *n* (%)	51(10.16)	4 (7.84)	14 (27.45)	22(43.14)	11(21.57)
Educational level					
Student, *n* (%)	97 (19.32)	69 (88.46)	27 (12.98)	1 (0.64)	0 (0)
Unemployed, *n* (%)	53 (10.56)	2 (2.56)	14 (6.73)	26 (16.67)	11 (18.33)
Active worker, *n* (%)	315 (62.75)	2 (2.56)	163 (78.37)	122 (78.21)	28 (46.67)
Retired, *n* (%)	21 (4.18)	0 (0)	0 (0)	3 (1.92)	18 (30)
All others, *n* (%)	16 (3.19)	5 (6.41)	4 (1.92)	4 (2.56)	3 (5)

for multiple testing when appropriate. Univariable and multi-variable logistic regression adjusted for age and sex with robust variance estimator adjusted for clustering within health clinics was used to evaluate the association between selected variables and test positivity. Prevalence odds ratios (OR) with 95% confidence intervals (CI) for the association were generated. All tests were conducted at a significance level of 0.05.

Results

Characteristics of study population

All patients of any age were eligible to participate in the study and were categorized by sex, age group and symptoms. Out of 510 enrolled individuals, 502 were included in the final analysis. Eight samples were excluded because the consent signature was missing (n=5) or they were from the same family (n=3) (Figure 1). The female/male ratio was 1.2 (274/228).

The median age of participants was 37.6 years (range 0–90 years), with 78 (16%) children and adolescents (aged 0–18 years). The majority of participants (72%) were in the 19–39-years and 40–59-years age groups. The youngest patient identified was 9 days old. Key characteristics of participants and recruitment by site are provided in Table 1. Prevalence is shown among participants at health clinics from three cities (Managua, León and Masaya).

Prevalence of LAMP-detectable SARS-CoV-2 infection

Among the 502 study participants, 93 tested positive for SARS-CoV-2 using the LAMP method, corresponding to prevalence of 19%. The prevalence of infection was examined among participants in four different age groups: 0–18, 19–39, 40–59 and ≥60 years (Table 1). Prevalence varied between the age groups, showing that the older (≥60 years) patients had the highest positive rate (27%). Of the 78 children enrolled in this cohort, 15 (19.2%) tested positive. In the overall sample, more females were tested than males (54.6% and 45.4%, respectively) (Table 2). The prevalence of infection was significantly higher among females than among males (P=0.03). Sixty of 274 (21.9%) female subjects tested positive, compared with 33 of 228 (14.5%) male subjects (P=0.03).

Symptoms of COVID-19 were reported by 29% of those in the overall population-screening group, and by 46% of SARS-CoV-2-positive participants (P<0.001). Additionally, 14% of participants who did not report symptoms (50/356) were LAMP-positive, compared with 29% of symptomatic individuals (43/146; P<0.001). Fifty-four percent of LAMP-positive subjects were asymptomatic, compared with 75% of LAMP-negative subjects. More than half of the asymptomatic subjects (52%) were aged <50 years. Among LAMP-positive individuals, males had a higher rate of asymptomatic infection than females (60.6% vs 50%, respectively). Reports of symptoms were less common among participants in the city of León, with only 7.5% presenting with COVID-19 symp-

Table 2
Severe acute respiratory syndrome coronavirus-2 loop-mediated isothermal amplification (LAMP) test results and medical history of study participants.

	Overall, *n* (%)[a] *n*=502	Positive LAMP,*n* (%)*n*=93	Negative LAMP,*n* (%)*n*=409	*P*-value[b]
Females	274 (54.58)	60 (64.52)	214 (52.32)	**0.03**
Males	228 (45.42)	33 (35.48)	195 (47.68)	**0.03**
Declared symptoms				
No symptoms	356 (70.92)	50 (53.76)	306 (74.82)	**<0.001**
At least one COVID-19 symptom	146 (29.08)	43 (46.24)	103 (25.18)	**<0.001**
Cough	69 (13.75)	27 (29.03)	42 (10.27)	**<0.001**
Sore throat	67 (13.35)	18 (19.35)	49 (11.98)	0.09
Headache	60 (11.95)	19 (20.43)	41 (10.02)	**0.02**
Fever	46 (9.16)	13 (13.98)	33 (8.07)	0.12
Body aches and discomfort	33 (6.57)	7 (7.53)	26 (6.36)	0.69
Asthenia	26 (5.18)	8 (8.60)	18 (4.40)	0.17
Diarrhea	24 (4.78)	4 (4.30)	20 (4.89)	0.80
Pain or pressure in the chest	17 (3.39)	7 (7.53)	10 (2.44)	0.07
Loss of smell	14 (2.79)	7 (7.53)	7 (1.71)	**0.04**
Loss of taste	11 (2.19)	5 (5.38)	6 (1.47)	0.11
Difficulty breathing	8 (1.59)	3 (3.23)	5 (1.22)	0.29
Pre-existing medical conditions				
At least one condition	210 (41.83)	43 (46.24)	167 (40.83)	0.34
Hypertension	86 (17.13)	21 (22.58)	65 (15.89)	0.15
Respiratory disease	32 (6.37)	7 (7.53)	25 (6.11)	0.63
Obesity	29 (5.78)	5 (5.38)	24 (5.87)	0.85
Diabetes	24 (4.78)	5 (5.38)	19 (4.65)	0.77
Cardiovascular disease	19 (3.78)	3 (3.23)	16 (3.91)	0.74
Cancer	12 (2.39)	4 (4.30)	8 (1.96)	0.29
Autoimmune diseases	9 (1.79)	2 (2.15)	7 (1.71)	0.78
Chronic obstructive pulmonary disease	5 (1.00)	2 (2.15)	3 (0.73)	0.36
Other characteristics				
Works mainly from home	124 (24.70)	13 (13.98)	111 (27.14)	**0.002**
Use public transport	130 (25.90)	29 (31.18)	101 (24.69)	0.22
Presence of a family member with COVID-19 symptoms at home	65 (12.95)	22 (23.66)	43 (10.51)	**0.005**
Reduced contact	107 (21.31)	24 (25.81)	83 (20.29)	0.27

COVID-19, coronavirus disease 2019.
[a] Percentages are calculated by column.
[b] Bold values are considered statistically significant.

toms during the study period (Table S2, see online supplementary material).

A summary of reported symptoms and their frequencies can be seen in Table 2. The most common symptoms reported by those who tested positive were cough (29%, *P*<0.001), headache (20%, *P*=0.02), sore throat (19%, *P*=0.09) and fever (14%, *P*=0.12). Loss of smell or taste was reported by 12 (13%, *P*=0.04) of those who tested LAMP-positive. Symptomatic individuals who tested positive were 2.7 times more likely to experience difficulty breathing or shortness of breath than those who tested negative (3.23 vs 1.22, respectively). Forty-two percent of the study participants admitted pre-existing health conditions in their medical history. However, among the overall SARS-CoV-2-positive cases, 46% had one or more health conditions, in contrast with 41% of those who tested negative (*P*=0.34) (Table 2). The most prevalent self-reported comorbidities among those who tested positive were hypertension (22.6%, *P*=0.15), respiratory diseases (7.5%, *P*=0.63), obesity (5.4%, *P*=0.85), diabetes (5.4%, *P*=0.77), cardiovascular diseases (3.2%, *P*=0.74) and cancer (4.3%, *P*=0.29).

Of 93 LAMP-positive cases, 22 (23.7%, *P*=0.005) had recent contact with known COVID-19 cases at home. Twenty-five percent of the participants reported working fully or partially from home. Of 130 individuals who reported using public transport, 29 (22.3%, *P*=0.22) were LAMP-positive.

Seroprevalence of SARS-CoV-2 antibodies

Of the 502 individuals in the study population, 191 tested positive for either IgG or IgM, representing overall seroprevalence of 38% (Table 3). Overall, 83 (16.5%) tested positive for IgM, and 184 (36.7%) tested positive for IgG.

The proportion of participants who tested positive varied by sex and age. The proportion of males positive for IgM (19.3%) was significantly higher than that of females (14.2%). However, the proportion testing positive for IgG was relatively similar (36.8% vs 36.5%). Within participants aged ≤18 years, 10 (13%) were SARS-CoV-2 seropositive for IgG, which was the lowest prevalence among all age groups. On the contrary, participants aged 19–39 and 40–59 years presented the highest seroprevalence for IgG (41.8% and 41.7%, respectively). For participants aged ≥60 years, IgG seroprevalence was 36.7%. In the case of IgM, the seropositive rates were 5.1%, 16.8%, 18% and 26.7%, respectively, for participants aged <18, 19–39, 40–59 and ≥60 years, respectively. The age group that presented the highest seropositivity rate for either IgG or IgM (at least one) was the 19–39-years age group (43.8%), followed by the 40–59-years age group (42.3%) (Table 3).

More than 10% of the study participants reported the death of a family member during the pandemic. Among those, 41% were seropositive for either IgG or IgM. However, seroprevalence was similar in people with or without a family member who died from COVID-19. One hundred and eighty-one participants believed that they acquired the disease during the first year of the pandemic, mainly between May and August 2020.

Association analysis

A number of important statistical associations were identified using simple Chi-squared contingency tables and two-tailed *P*-values with appropriate correction for multiple tests (Table S3, see online supplementary material). Females were more likely to be LAMP-positive than males (22% vs 14%, respectively; Chi-squared 4.54, *P*=0.03), but less likely to be seropositive (31% vs 44%, respectively; Chi-squared 9.34, *P*=0.002). Also, children were less likely to be seropositive than other age groups (*P*<0.001). There was a trend towards an association between being LAMP-positive and older age, with participants aged ≥60

Table 3
Seroprevalence of severe acute respiratory syndrome coronavirus-2 among study participants.

Category	IgG positive	IgG seroprevalence, %	IgM positive	IgM seroprevalence, %	IgG/IgM positive[a]	IgG/IgM seroprevalence, %
Sex						
Female, *n*=274	100	36.50	39	14.23	103	37.59
Male, *n*=228	84	36.84	44	19.30	88	38.60
Age, years						
All ages, *n*=502	184	36.65	83	16.53	191	38.05
0–18, *n*=78	10	12.82	4	5.13	12	15.38
19–39, *n*=208	87	41.83	35	16.83	91	43.75
40–59, *n*=156	65	41.67	28	17.95	66	42.31
≥60, *n*=60	22	36.67	16	26.67	22	36.67
Other characteristics						
Suspected COVID-19 during the previous 11 months of the pandemic, *n*=181	113	62.43	54	29.83	114	62.98
Had COVID-19 symptoms in the last 30 days, *n*=129	28	21.71	19	14.73	33	25.58
Death of a close family member from COVID-19, *n*=59	23	38.98	11	18.64	24	40.68
Hospital or clinic location						
Managua, *n*=451	167	37.03	76	16.85	173	38.36
León and Masaya, *n*=51	17	33.33	7	13.73	18	35.29

COVID-19, coronavirus disease 2019; Ig, immunoglobulin.
[a] Either IgM or IgG positive.

years more likely to be LAMP-positive than younger subjects; however, this did not quite reach significance (P=0.06).

Participants with any symptoms were more likely to be LAMP-positive than asymptomatic participants (P<0.001), but the negative predictive value of being asymptomatic was only 75%. Among common symptoms, cough was most strongly associated with being LAMP-positive (P<0.001), but the positive predictive value was only 49%. Loss of smell was the symptom with the highest positive predictive value (50%), and was significantly associated with being LAMP-positive (P=0.007, Fisher's exact for small sample number). When applying Bonferroni's correction for multiple tests, no pre-existing condition was significantly associated with being LAMP-positive. Having a family member with symptoms was associated with being LAMP-positive (P=0.0007).

A logistic regression model was used to further assess the factors associated with infection positivity. Table 4 shows associations between symptoms and being LAMP-positive.

In univariable analyses, the odds of LAMP positivity were significantly higher in participants with at least one symptom (OR 2.55, 95% CI 1.89–3.45), cough (OR 3.57, 95% CI 2.65–4.81), sore throat (OR 1.76, 95% CI 1.40–2.22), headache (OR 2.30, 95% CI 1.88–2.82), fever (OR 1.85, 95% CI 1.14–3.01), asthenia (OR 2.04, 95% CI 1.60–2.62), pain or pressure in the chest (OR 3.25, 95% CI 2.42–4.36), loss of smell (OR 4.67, 95% CI 2.85–7.65), loss of taste (OR 3.82, 95% CI 2.60–5.59) and difficulty breathing (OR 2.69, 95% CI 1.94–3.74). The significant level of associations was consistent after adjusting for age and sex. The complete analysis is presented in Table S4 (see online supplementary material).

Interpreting the prevalence data from LAMP and serology testing combined

To better interpret the results of both diagnostic tests in terms of infection prevalence, study participants were sorted into four categories: LAMP-negative/seronegative; LAMP-positive/seronegative; LAMP-positive/seropositive; and LAMP-negative/seropositive (Table 5). Accordingly, 13.8% of the study participants had evidence of an active recent infection (LAMP-positive/seronegative), and an additional 4.8% had evidence of a recent infection (LAMP-positive/seropositive). Furthermore, 48.2% of the participants had not been exposed to SARS-CoV-2 (LAMP-negative/seronegative), and 51.8% had SARS-CoV-2 infection (either LAMP-positive or seropositive).

Discussion

Sample testing of patients who were seeking care for issues unrelated to COVID-19 was conducted to contribute to limited knowledge regarding the prevalence of current infection and presence of antibodies to SARS-CoV-2 in Nicaragua. This was accomplished through the COVID-19 network in eight participating urban health clinics between 22 February and 19 March 2021, 1 year into the pandemic. Several important observations were made.

The main finding of the study was that the prevalence rates for current infection (14%, LAMP-positive/seronegative) and past infection (38% seropositive) were fairly high. While 19% of the study participants were LAMP-positive, the prevalence of current infection was estimated to be 14% by examining the fraction of people who were LAMP-positive but seronegative. This finding, along with the overall seropositivity rate of 38%, reveals that by the end of the study period (mid-March 2021), more than half (52%) of the study sample had been infected with SARS-CoV-2; this is a very high percentage, but does not reach the collective or herd immunity threshold of 60–80% needed to block most SARS-CoV-2 transmission (Gomes et al., 2020; Randolph and Barreiro, 2020).

The majority (54%) of LAMP-positive participants were asymptomatic, with males found to be more likely to be asymptomatic than females (60.6% vs 50%, respectively). Females were more likely to be LAMP-positive than males, but were less likely to be seropositive. One

Table 4
Univariable and multivariable analyses of association between clinical symptoms and positive loop-mediated isothermal amplification result for severe acute respiratory syndrome coronavirus-2.

Characteristics	Prevalence odds ratio	95% confidence interval	Adjusted prevalence odds ratio[a]	95% confidence interval
Symptoms				
At least one COVID-19 symptom, *n*=146	**2.55**	**1.89–3.45**	**2.98**	**2.15–4.12**
Cough, *n*=69	**3.57**	**2.65–4.81**	**3.83**	**2.86–5.10**
Sore throat, *n*=67	**1.76**	**1.40–2.22**	**2.05**	**1.68–2.50**
Headache, *n*=60	**2.30**	**1.88–2.82**	**2.42**	**1.99–2.96**
Fever, *n*=46	**1.85**	**1.14–3.01**	**2.34**	**1.39–3.95**
Body aches and discomfort, *n*=33	1.20	0.69–2.08	1.15	0.60–2.23
Asthenia, *n*=26	**2.04**	**1.60–2.62**	**2.12**	**1.66–2.70**
Diarrhoea, *n*=24	0.87	0.68–1.12	0.98	0.72–1.33
Pain or pressure in the chest, *n*=17	**3.25**	**2.42–4.36**	**3.00**	**2.32–3.86**
Loss of smell, *n*=14	**4.67**	**2.85–7.65**	**4.27**	**2.73–6.69**
Loss of taste, *n*=11	**3.82**	**2.60–5.59**	**4.10**	**2.82–5.96**
Difficulty breathing, *n*=8	**2.69**	**1.94–3.74**	**2.64**	**1.91–3.63**

COVID-19, coronavirus disease 2019; *n*, sample size.
[a] Adjusted for age and sex.Bold values are considered statistically significant.

Table 5
Determining infection status from loop-mediated isothermal amplification (LAMP) and serology testing combined.

Category	Infection status	Number(*n*=502)	Prevalence(%)
LAMP-negative/seronegative	Not exposed	242	48.21
LAMP-positive/seronegative	Current infection	69	13.75
LAMP-positive/seropositive	Recent infection	24	4.78
LAMP-negative/seropositive	More distant infection	167	33.27

possible hypothesis is that more males than females were infected in an earlier pandemic wave.

Participants with any symptoms were more likely to be LAMP-positive than asymptomatic participants, and loss of smell was the symptom with the highest positive predictive value. Loss of smell was significantly associated with being LAMP-positive, as has been established in other settings (Lechien et al., 2020). Forty-six percent of LAMP-positive cases had at least one underlying medical condition, with hypertension, obesity and diabetes being the most prevalent; these conditions are known to increase a person´s risk of severe COVID-19.

Seroprevalence was also very high at almost 40%; however, this was still insufficient to provide natural collective immunity and reduce the spread of the virus. This explains why active infection, estimated by LAMP detection of SARS-CoV-2 RNA, was also high (14%). Additionally, almost 17% of the study population was IgM positive, confirming recent infection. In this uncontrolled sample, more than half of the participants showed evidence of current or past infection.

Although one cannot rigorously extrapolate the findings from the studied sample to Nicaragua as a whole, the data, obtained from testing for SARS-CoV-2 infection in three urban populations, provide an opportunity to compare the results of Nicaragua's response to COVID-19 with those of the more severe approaches of neighbouring countries. The SARS-CoV-2 pandemic has had a devastating impact in Latin America (Undurraga et al., 2021; Mendoza, 2021), while implementing different virus control policies. Considerable variation in seroprevalence exists throughout the region (Pan American Health Organization, 2021). Seroprevalence found in this study was similar to the published prevalence for various Latin American countries at different times during the pandemic (Huamaní et al., 2020; Instituto Nacional de Salud Colombia, 2020; Silva et al., 2020; Del Brutto et al., 2021). Also, an unpublished study carried out in October 2020 in the city of León, Nicaragua found slightly lower seroprevalence (34%) in the overall population (González et al., 2021), and a recent publication suggested widespread and sustained community viral transmission in Nicaragua (Huete-Pérez et al., 2021b).

A key strength of this study is that it provides important, urgently needed information to contribute to understanding of the COVID-19 epidemic in Nicaragua. The data were obtained using a combination of two complementary assays: a nucleic acid detection technique (LAMP) and a widely available lateral flow immunoassay test.

The study data may not accurately reflect the national prevalence of SARS-CoV-2 in Nicaragua. The conclusions are limited to the non-controlled sample used. Also, most participants were from Managua, the capital of Nicaragua, although the authors were also able to enrol participants from León and Masaya, together representing the three most densely populated cities in the country. It is important not to overinterpret the statistics. While providing a fairly representative sample of people visiting health facilities, this is not a random sample of the population at large. Visitors may have been more inclined to participate in the study if they feared past exposure to an infected individual or had experienced symptoms.

A significant limitation of this study is that some heterogeneity was detected between the populations in the enrolled clinics that was out of the authors' control. Although the study design did not intentionally select these clinics based on any conceivable differences, it is important to point out that there were large differences in both demographic characteristics and test positivity between the enrolled clinics. Therefore, given the low number of results per site and the reduced power of the comparisons between clinics, it is not possible to make convincing inferences on nationwide prevalence, which requires further investigation.

Data from this survey may underestimate the true number of SARS-CoV-2 infections, as some asymptomatic individuals may not seroconvert (Ejazi et al., 2021), while others may have been tested prior to seroconversion or when antibodies had waned below the detection limit (Self et al., 2020). However, by using LAMP as a complementary nucleic acid technique, it was possible to identify most active infections. This strategy also helped identify asymptomatic individuals, who represented 54% of the LAMP-positive cases in this study. While acknowledging these limitations, the data provided in this study are critical to estimate and monitor the growing burden of COVID-19.

Together these results indicate that over the first year of the pandemic, SARS-CoV-2 infection may have reached a large segment of the Nicaraguan population, and that during the study period, the infection was still widespread and clearly related to the Nicaraguan Government's

response to the pandemic, which permitted the virus to run its course largely unchecked. Failure to take appropriate measures to control the epidemic may have played a leading role in runaway community transmission. The health crisis was additionally compounded by insufficient testing, resulting in the lack of information on the extent of the disease in Nicaragua. One important implication of the data is that symptom-based screens are likely to be ineffective as there are so many asymptomatic LAMP-positive individuals. Also, the data highlight the importance of strict infection control practices at these clinics, without which susceptible individuals visiting the clinics for non-COVID-related issues could be at significant risk of infection.

This study highlights the need to produce robust data to establish the true extent of SARS-CoV-2 infection in Nicaragua and to guide appropriate interventions. It further underscores the urgent need for extensive testing as part of a coherent scientifically sound strategy (Hasell et al., 2020; Salathé et al., 2020; Seidu et al., 2020; World Health Organization, 2020) to effectively respond to the COVID-19 pandemic. The combination of LAMP assay with serological testing, as shown in this study, may be employed at the point-of-care and in epidemiological control – an approach that has been used effectively in large epidemiological studies (Mlcochova et al., 2020; Sidiq et al., 2020; Zhang et al., 2020; Kevadiya et al., 2021; Pape et al., 2021; Tönshoff et al., 2021).

Conclusions

These data provide seminal insight into the pandemic's progress in Nicaragua, specifically the prevalence of current SARS-CoV-2 infection. They are important for clinicians and public health workers, and for regional health organizations seeking to understand the rapid viral spread within vulnerable communities in the absence of effective policies to confront the SARS-CoV-2 pandemic.

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Conflict of interest statement

None declared.

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Ethical approval

This study was reviewed and approved by the Ethics Committee of the University of Central America (#WB-2021-02-20), and conducted according to the principles expressed in the Declaration of Helsinki. Written research authorization and informed consent were obtained from all participants or the parents/legal guardians of all participating children.

Author contributions

JAH-P conceived the study and its design, wrote the original draft of the manuscript, accepts full responsibility for the work and/or the conduct of the study, had access to the data, takes responsibility for the integrity of the data and accuracy of the analysis, and controlled the decision to publish.

CC-R and LP-M contributed to data collection, and organized and entered data.

RCC and BH contributed to writing, statistical analyses and interpretation.

SS and CQ contributed to data collection, analyses and interpretation.

AH contributed to the study design, data analyses and interpretation, and writing.

All authors contributed to the final drafting of the manuscript, and have seen and approved the submitted version of this manuscript.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijregi.2021.12.013.

References

Associated Press. PAHO waits for Nicaragua to approve visit by virus experts. The Washington Post 2 July 2020. Available at: https://www.washingtonpost.com/world/the_americas/paho-waits-for-nicaragua-to-approve-visit-by-virus-experts/2020/07/21/af31ac9c-cba5-11ea-99b0-8426e26d203b_story.html (accessed 7 May 2021).

CitizenObservatory. [Citizen Observatory COVID-19 weekly report in Nicaragua 19/11/2020 to 25/11/2020]. Citizen Observatory, Managua, Nicaragua; 2020. Available at: https://observatorioni.org/informes-notas-y-semanales/ (accessed 20 October 2021).

Dao Thi VL, Herbst K, Boerner K, Meurer M, Kremer LPM, Kirrmaier D, et al. A colorimetric RT-LAMP assay and LAMP-sequencing for detecting SARS-CoV-2 RNA in clinical samples. Sci Transl Med 2020;12:eabc7075.

Del Brutto OH, Costa AF, Mera RM, Recalde BY, Bustos JA, García HH. SARS-CoV-2 in rural Latin America. A population-based study in Coastal Ecuador. Clin Infect Dis 2021;73:314–17.

Ejazi SA, Ghosh S, Ali N. Antibody detection assays for COVID-19 diagnosis: an early overview. Immunol Cell Biol 2021;99:21–33.

Gomes MGM, Corder RM, King JG, Langwig KE, Souto-Maior C, Carneiro J, et al. Individual variation in susceptibility or exposure to SARS-CoV-2 lowers the herd immunity threshold. MedRxiv 2020 2020.04.27.20081893.

González F, Vielot NA, Sciaudone M, Toval-Ruíz C, Premkumar L, Gutierrez L, et al. Seroepidemiology of SARS-CoV-2 infections in an urban Nicaraguan population. MedRxiv 2021 2021.02.25.21252447.

Hasell J, Mathieu E, Beltekian D, Macdonald B, Giattino C, Ortiz-Ospina E, et al. A cross–country database of COVID-19 testing. Sci Data 2020;7:345.

Huamaní C, Velásquez L, Montes S, Miranda-Solis F. Propagation by COVID-19 at high altitude: Cusco case. Respir Physiol Neurobiol 2020;279.

Huete-Pérez JA, Cabezas-Robelo C, Páiz-Medina L, Hernández-Álvarez CA, Quant–Durán C, McKerrow JH. First report on prevalence of SARS-CoV-2 infection among health-care workers in Nicaragua. PLoS One 2021a;16.

Huete-Pérez JA, Ernst KC, Cabezas-Robelo C, Páiz-Medina L, Silva S, Huete A. Prevalence and risk factors for SARS-CoV-2 infection in children with and without symptoms seeking care in Managua, Nicaragua: results of a cross-sectional survey. BMJ Open 2021b;11.

Instituto Nacional de Salud Colombia. [Seroprevalence of SARS-CoV-2 during pandemic in Colombia: country study]. Instituto Nacional de Salud Colombia, Bogotá, Colombia; 2020 Available at: https://www.ins.gov.co/Direcciones/Investigacion/seroprevalencia-covid accessed 7 May 2021.

Karlinsky A, Kobak D. The World Mortality Dataset: tracking excess mortality across countries during the COVID-19 pandemic. MedRxiv 2021 2021.01.27.21250604.

Kevadiya BD, Machhi J, Herskovitz J, Oleynikov MD, Blomberg WR, Bajwa N, et al. Diagnostics for SARS-CoV-2 infections. Nat Mater 2021;20:593–605.

Lechien JR, Chiesa-Estomba CM, De Siati DR, Horoi M, Le Bon SD, Rodriguez A, et al. Olfactory and gustatory dysfunctions as a clinical presentation of mild-to-moderate forms of the coronavirus disease (COVID-19): a multicenter European study. Eur Arch Oto-Rhino-Laryngol 2020;277:2251–61.

Mendoza J. Latin America: COVID-19 deaths 2021, by country. Baltimore, MD: Johns Hopkins University; 2021 Available at https://www.statista.com/statistics/1103965/latin-america-caribbean-coronavirus-deaths/ accessed 7 May 2021.

Mlcochova P, Collier D, Ritchie A, Assennato SM, Hosmillo M, Goel N, et al. Combined point-of-care nucleic acid and antibody testing for SARS-CoV-2 following emergence of D614G spike variant. Cell Reports Med 2020;1.

Pan American Health Organization. SARS-COV-2 Seroprevalence studies in Latin America and the Caribbean. Washington, DC: PAHO/WHO; 2021 Available at: https://ais.paho.org/phip/viz/COVID-19Seroprevalence.asp accessed 7 May 2021.

Pape C, Remme R, Wolny A, Olberg S, Steffen Wolf, Cerrone L, et al. Microscopy-based assay for semi-quantitative detection of SARS-CoV-2 specific antibodies in human sera: a semi-quantitative, high throughput, microscopy-based assay expands existing approaches to measure SARS-CoV-2 specific antibody levels in human sera. Bioessays 2021;43.

PRONicaragua. [Demographic profile. Population distribution by department Nicaragua 2020]. Managua: PRONicaragua; 2020 Available at: http://www.pronicaragua.gob.ni/es/informes-y-presentaciones/41-perfil-demogr%C3%A1fico-2020/ accessed 7 May 2021.

Randolph HE, Barreiro LB. Herd immunity: understanding COVID-19. Immunity 2020;52:737–41.

Rodino KG, Espy MJ, Buckwalter SP, Walchak RC, Germer JJ, Fernholz E, et al. Evaluation of saline, phosphate-buffered saline, and minimum essential medium as potential alternatives to viral transport media for SARS-CoV-2 testing. J Clin Microbiol 2020;58:e00590 -20.

Salathé M, Althaus CL, Neher R, Stringhini S, Hodcroft E, Fellay J, et al. COVID-19 epidemic in Switzerland: on the importance of testing, contact tracing and isolation. Swiss Med Wkly 2020;150:w20225.

Seidu A-A, Hagan JE, Ameyaw EK, Ahinkorah BO, Schack T. The role of testing in the fight against COVID-19: current happenings in Africa and the way forward. Int J Infect Dis 2020;98:237–40.

Self WH, Tenforde MW, Stubblefield WB, Feldstein LR, Steingrub JS, Shapiro NI, et al. Decline in SARS-CoV-2 antibodies after mild infection among frontline health care personnel in a multistate hospital network –12 states, April–August 2020. MMWR Morb Mortal Wkly Rep 2020;69:1762–6.

Sidiq Z, Hanif M, Dwivedi KK, Chopra KK. Benefits and limitations of serological assays in COVID-19 infection. Indian J Tuberc 2020;67:S163–6.

Silva AAM da, Lima-Neto LG, Azevedo C de MPES de, Costa LMM da, Bragança MLBM, Barros Filho AKD, et al. Population-based seroprevalence of SARS-CoV-2 and the herd immunity threshold in Maranhão. Rev Saude Publ 2020;54:131.

Tang Y-W, Schmitz JE, Persing DH, Stratton CW. Laboratory diagnosis of COVID-19: current issues and challenges. J Clin Microbiol 2020;58:e00512–20.

Tönshoff B, Müller B, Elling R, Renk H, Meissner P, Hengel H, et al. Prevalence of SARS-CoV-2 infection in children and their parents in Southwest Germany. JAMA Pediatr 2021. doi:10.1001/jamapediatrics.2021.0001.

Undurraga EA, Chowell G, Mizumoto K. COVID-19 case fatality risk by age and gender in a high testing setting in Latin America: Chile, March–August 2020. Infect Dis Poverty 2021;10:11.

World Health Organization. Critical preparedness, readiness and response actions for COVID-19. Geneva: WHO; 2020 Available at: https://www.who.int/publications/i/item/critical-preparedness-readiness-and-response-actions-for-covid-19 accessed 7 May 2021.

Yan C, Cui J, Huang L, Du B, Chen L, Xue G, et al. Rapid and visual detection of 2019 novel coronavirus (SARS-CoV-2) by a reverse transcription loop-mediated isothermal amplification assay. Clin Microbiol Infect 2020;26:773–9.

Zhang W, Du R-H, Li B, Zheng X-S, Yang X-L, Hu B, et al. Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes. Emerg Microbes Infect 2020;9:386–9.

Zhang Y, Odiwuor N, Xiong J, Sun L, Nyaruaba RO, Wei H, et al. Rapid molecular detection of SARS-CoV-2 (COVID-19) virus RNA using colorimetric LAMP. MedRxiv 2020a 2020.02.26.20028373.

Zhang Y, Ren G, Buss J, Barry AJ, Patton GC, Tanner NA. Enhancing colorimetric loop-mediated isothermal amplification speed and sensitivity with guanidine chloride. Biotechniques 2020b. doi:10.2144/btn-2020-0078.