

Seroprevalence of IgM and IgG anti-SARS-COV-2 and associated factors among agricultural workers in Colombia

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Abstract

Background: The population of South America has been severely affected by the COVID-19 pandemic. In this region, during the year 2020, high seroprevalence percentages were reported, which have been associated with the socioeconomic characteristics of the population, mainly in urban areas. However, a relative lack of information on the dynamics of the pandemic in rural areas of these countries, where the population is more vulnerable, is still present. This study determined antibody prevalence against SARS-CoV-2 in urban and rural food producing workers in Colombia.

Methods: A total of 1242 workers, urban and rural, linked to poultry, dairy, and meat production and supply chains, were analyzed through a sociodemographic survey and two serological tests against S and N proteins of SARS-CoV-2.

Results: 78.7% were male. 50.9% of the participants were rural inhabitants, with an average age of 40.9 years old. 39.2% had IgM and IgG against SARS-CoV-2 S protein and 31.3% against N protein for the same virus; 83.6% had not been tested with an RT-PCR test for COVID-19 and 75.7% did not report symptoms related to the disease. The associated risk factors were low education, OR: 1.46, greater number of cohabitants, OR: 1.36, and contact with people infected with COVID-19, OR: 2.03.

Conclusions: The seroprevalences found suggest an important interconnectivity between rural and urban areas, where asymptomatic subjects and sociodemographic factors facilitate the virus' spread in the population.

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Keywords: Agricultural workers, Colombia, COVID-19, SARS-CoV-2 antibody levels

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Introduction

The South American population has been severely affected by COVID-19, with more than 40 million confirmed cases as of January 2022 [1]. This region, relative early in the pandemic, reported the highest seroprevalence to SARS-CoV-2 in the world, with Iquitos, Peru, (70.0%) [2], in Manaus (Brazil) (76.0%) [3] and Buenos Aires, Argentina, (53.4%) [4]. In Colombia, the first national seroprevalence survey, in September 2020, found seroprevalence between 68.0% and 27.0% [5]. The second wave of the

COVID-19 pandemic was reported in January 2021, and the third wave started in the last weeks of April, with the highest number of cases and deaths reported, reaching 4,886,897 confirmed cases by August of 2021 [6]. High seroprevalence in Colombia seems to have been associated with contact with COVID-19 asymptomatic patients and sociodemographic conditions [7–11]. Even so, in Colombia, there is little evidence of the virus's mode of spread in urban and rural communities [12]. Therefore, we aimed to determine the prevalence of antibodies against SARS-CoV-2 in urban and rural food-producing workers in Colombia.

Material and methods

Study design and sampling

A cross-sectional epidemiological study was conducted in rural and urban communities linked to three animal protein

production and supply chains in Colombia (dairy, livestock/meat and milk and poultry value chains). The sample size was calculated for an expected proportion between 14.6% and 50.4% of infected individuals (RT-PCR positive for SARS-CoV-2) reported by the National Health Institute (INS) of Colombia, in each region where the respective value chains were located [6]. The size of the sample was determined with a confidence level of 95% and an expected error of 5%, including 1262 adults. *Inclusion criteria:* inhabitants of rural and urban areas, who are adults and workers, as well as family members, linked to the selected production chains. *Exclusion criteria:* people vaccinated with one or two doses of COVID-19. Non-probabilistic sampling was carried out through the snowball method, between March and June 2021 (a period corresponding to the third peak of the pandemic in Colombia) by registered nurses from Health Service Providers Institutions.

Ethics statement

All participants signed the informed consent endorsed by the Ethics Committee from the Health Sciences Faculty through folio No. 065, complying with the 1975 Declaration of Helsinki.

Data collection and laboratory processing

A self-administered questionnaire was distributed by letter to participants' workplaces, to collect the sociodemographic data and health status in the last six months. Following the biosafety protocol, a venous blood sample was collected without anticoagulant, which was transported to the laboratory in an ice chamber within 12 hours. The serum was separated by centrifugation (1200 rpm × 10 min) and stored at -70 °C, until processing.

Serological tests

The COV2T (Siemens Healthineers, USA) test was carried out, for IgG and IgM detection against the RBD region of the SARS-CoV-2 S protein in human serum and plasma. The test has a 100% sensitivity after 7 days of the presence of symptoms and a 99.8% clinical specificity (95% CI 99.4-99.9%) [13]. *In-house serological test:* LaSalleCoV2N for the detection of IgG and IgM antibody against SARS-CoV-2 N_r protein, produced in BL21 (DE3) cells (donated by Universidad Nacional de Colombia) transformed with pET-28a (+) Vector using the SARS-COV-2 nucleoprotein gene, donated by BEI Resources, NIAID, NIH: NR-53507 (USA). Immunoplates (SPL Life Sciences, Korea) were sensitized with 0.2 µg/mL of N_r protein. Sera were added in duplicate at 100-fold dilution and secondary antibodies: Goat Anti-Human IgG F(ab)² (Invitrogen, USA) and Goat Anti-Human IgM F(ab)² (SouthernBiotech, USA), HRP conjugated (each at 5000-fold dilution). The reaction was revealed with luminol (ABCAM, USA) and read on a luminoskan (Thermo

Fischer, USA). Each trial used the international standard S321834 NIBSC 1000 IU/mL, donated by the WHO Solidarity II group. The LaSalleCoV2N test in symptomatic positive RT-PCR patients versus pre-pandemic sera has a 91.3% sensitivity (95% CI 86.1-94.8%) and a 93.0% specificity (95% CI 86.8-97.0%). In asymptomatic positive RT-PCR patients, it has a 70.4% sensitivity (95% CI 58.4-80.7%) and a 93.0% specificity (95% CI 86.8-97.0%).

Statistical analysis

Descriptive analysis included sociodemographic and health variables. The final seroprevalence estimates were adjusted for the COV2T test sensitivity and specificity. Student's *t* and Chi-square tests were used to determine the difference significance in the bivariate analyses. Multivariate analyses were carried out using logistic regression models. The quality of logistic regression models was compared by using Akaike Information Criterion (AIC). The model with the lowest AIC value was considered as the best fit to data. The other selection criterion was based on the Pseudo R² values of the model that slightly better explain the variability of data compared to the other models. This is the statistical model that was used:

$$\begin{aligned} \text{Logit}(\text{SARS-CoV-2 presence}) = & \beta_0 + \beta_{\text{education level}} \\ & + \beta_{\text{contact people with COVID-19}} \\ & + \beta_{\text{number of cohabitants}} + \beta_{\text{age}} \\ & + \beta_{\text{use of bus transport}} \\ & + \beta_{\text{live with health personnel}} \\ & + \beta_{\text{COVID-19 compatible symptoms}} \\ & + \text{error} \end{aligned}$$

For each variable, odds ratios (OR) and their corresponding 95% confidence intervals (95% CI) were calculated. In all cases, a *P* < 0.05 value was considered statistically significant. The analyses were conducted with the R software, version 4.0.2 (The R Foundation for Statistical Computing).

Results

A total of 1242 adults linked to the dairy, livestock/meat and milk, and poultry chains were surveyed. The average age of the participants was 40.9 years old (SD ± 13.0) (Dairy chain: 38.8 ± 13.9; Poultry chain: 36.8 ± 12.1; livestock/meat and milk chain: 36.0 ± 12.3). The greater number of workers were male, especially in the poultry chains (78.5%) and livestock/meat and milk chain (70.7%). Most of the workers had some degree of secondary education, with the lowest level of schooling being found in the poultry chain. The motorcycle was the most used

means of transport, mainly in livestock/meat and milk chain (70.3%). Households with the highest number of inhabitants were found in the poultry chain (Table 1). 83.6% of the participants did not have a confirmed COVID-19 diagnosis by RT-PCR. The poultry chain showed the highest percentage of workers without a confirmatory diagnosis for this infection (92.8%). 75.7% of the participants did not report any disease-related symptoms in the previous 6 months, and the poultry chain had the highest percentage of asymptomatic workers (83.1%) for COVID-19 (Table 2).

At the sampling date, it was found that 39.2% of the subjects had antibodies against the SARS-CoV-2 S protein (COV2T), and 31.3% against the N protein (LaSalleCoV2N). In the dairy chain, 33.3% of the participants had antibodies against the virus S protein, and 34.5% had antibodies against the N protein. Seropositivity percentages were similar among the inhabitants of rural and urban areas. 55.4% of the workers in the livestock/meat and milk chain had antibodies against S protein and only 17.8% had antibodies against N protein. No correlation was found between the two serological tests in this community. For the poultry chain, 40.7% of people had antibodies against S protein and 36.6% against N protein. In urban areas, seropositivity percentages against S protein were higher (Fig. 1).

Of the total participants, 50.9% were rural inhabitants and 55.5% worked in this area. In rural workers, COVID-19 seropositivity was significantly associated with a low educational level ($p < 0.001$) and also being older than 50 years old ($p = 0.033$). Urban inhabitants presented higher seropositivity

to SARS-CoV-2 and this was significantly associated with contact with COVID-positive people ($p = 0.011$), inter-municipal travel ($p = 0.017$) and greater number of cohabitants ($p = 0.044$) (Table 3).

For the multivariate analysis, the results of the comparison between the models show that the best model had the AIC value (152.1) and ($R^2 = 0.43$). Multivariate analysis revealed that the lowest educational level, OR: 1.46 (95% CI 1.05-2.03, $p = 0.027$), a greater number of inhabitants in the household, OR: 1.36 (95% CI 1.01 - 1.85, $p = 0.047$), and having had contact with people infected with COVID-19, OR: 2.03 (95% CI 1.32 - 3.11, $p = 0.001$) predispose to infection from SARS-CoV-2.

Statistical analysis by value chain showed that many variables were associated with COVID-19 seropositivity in the dairy chain: low educational level ($p = 0.002$), having contact with people diagnosed with the disease ($p = 0.011$), the use of public transportation ($p = 0.043$) and living with more than 4 people ($p < 0.001$) (Table 4).

Discussion

This research, based on rural and urban food-producing communities in Colombia, found higher seropositivity to SARS-CoV-2 than that reported in the national seroepidemiological study [5], which was to be expected since the beginning of the sampling matched the third wave of the COVID-19 pandemic in

TABLE 1. Sociodemographic characteristics of the people linked to the food production chain in Colombia

Sociodemographic characteristics	Dairy chain		Livestock/meat and milk chain		Poultry chain		Total	
	n	(%)	n	(%)	n	(%)	n	(%)
Total number of participants	544	100	195	100	503	100	1242	100
Gender								
Male	291	53.5	138	70.7	395	78.5	824	66.3
Occupation								
Farm workers	228	41.9	106	54.4	365	72.6	699	56.3
Employees of company/plant	90	16.5	67	34.4	103	20.5	260	20.9
Student	16	2.9	8	4.1	7	1.4	31	2.5
Unknown	210	38.6	14	7.2	28	5.6	252	20.3
Educational level								
No school education	8	1.5	1	0.5	10	2.0	19	1.5
Elementary school	57	10.5	26	13.3	134	26.6	217	17.5
Middle and high school	134	24.6	82	42.1	180	35.8	396	31.9
Technician ^a	53	9.7	35	18.0	79	15.7	167	13.4
Professional	51	9.4	44	22.6	87	17.3	182	14.6
Unknown	241	44.3	8	4.1	0	0.0	249	20.0
Household								
Urban	113	20.8	117	60.0	228	45.3	458	36.9
Rural	283	52.0	77	39.5	272	54.1	632	50.9
ND	148	27.2	1	0.5	3	0.6	152	12.2
Number of cohabitants								
<4 persons	132	24.3	121	62.1	231	45.9	484	38.9
> = 4 persons	152	27.9	68	34.8	247	49.1	467	37.6
Unknown	260	47.8	6	3.1	3	0.6	269	21.7
Transport								
Car	87	16.0	36	18.5	89	17.7	212	17.1
Motorcycle	89	16.4	137	70.3	297	59.0	523	42.1
Bus	52	9.6	1	0.5	35	7.0	88	7.1
Bicycle/walking	118	21.7	11	5.6	53	10.5	182	14.7
Others	7	1.3	6	3.1	24	4.8	37	3.0
Unknown	191	35.1	4	2.2	5	1.0	200	16.1

^aTechnician: 1–2 years of studies, Professional: 4-5 years.

TABLE 2. Variables related to the COVID-19 pandemic in workers linked to food production chains in Colombia

		Dairy chain		Livestock/meat and milk chain		Poultry chain		Total	
		n	%	n	%	n	%	n	(%)
		COVID diagnosis	Yes	19	3.5	18	9.2	34	6.8
	No	395	72.6	176	90.3	467	92.8	1038	83.6
	Unknown	130	23.9	1	0.5	2	0.4	133	10.7
Contact with COVID-positive people	Yes	20	3.7	28	14.4	64	12.7	112	9.0
	No	345	63.4	164	84.1	435	86.5	944	76.0
	Unknown	179	32.9	3	1.5	4	0.8	186	15.0
Live with health workers	Yes	7	1.3	9	4.6	18	3.6	34	2.7
	No	362	66.5	184	94.4	483	96.0	1029	82.9
	Unknown	175	32.2	2	1.0	2	0.4	179	14.4
Symptoms of COVID-19 previous 6 months	Yes	46	8.5	32	16.4	78	15.5	156	12.6
	No	314	57.7	160	82.1	418	83.1	892	71.8
	Unknown	184	33.8	3	1.5	7	1.4	194	15.6

the country. In urban inhabitants, seropositivity to the virus was significantly associated with variables that reflect the dynamics of virus expansion in cities, overcrowding in homes, contact with COVID-positive people, and inter-municipal mobility [14–16]. The only variables associated with seropositivity in rural residents were a low educational level and being over 50 years old. Given that the level of education was similar in the two areas, it is possible that city inhabitants have an improved adherence to self-care protocols due to the ease of access to technologies of information and communication. Other countries have identified the same risk factors associated with COVID-19 prevalence and mortality in rural areas [17–20]. We confirm the importance of sociodemographic factors in the

virus’s transmissibility and disease severity in countries with high inequity [21–23].

According to the value chain, in the dairy and poultry chain many sociodemographic and health conditions were significantly associated with seropositivity to SARS-CoV-2 in the workers; this happens in part due to the structure of these chains. Most dairy and poultry farms are relatively small, close to cities, and the owners and/or workers live in the same place where they work, reducing the rural-urban interface. Furthermore, we show a high (35.5%) seroprevalence to SARS-CoV-2 in rural areas, indicating that the disease had a relatively early and fast widespread in Latin America rural population [18,19,24,25]. These might be explained by a lack of state

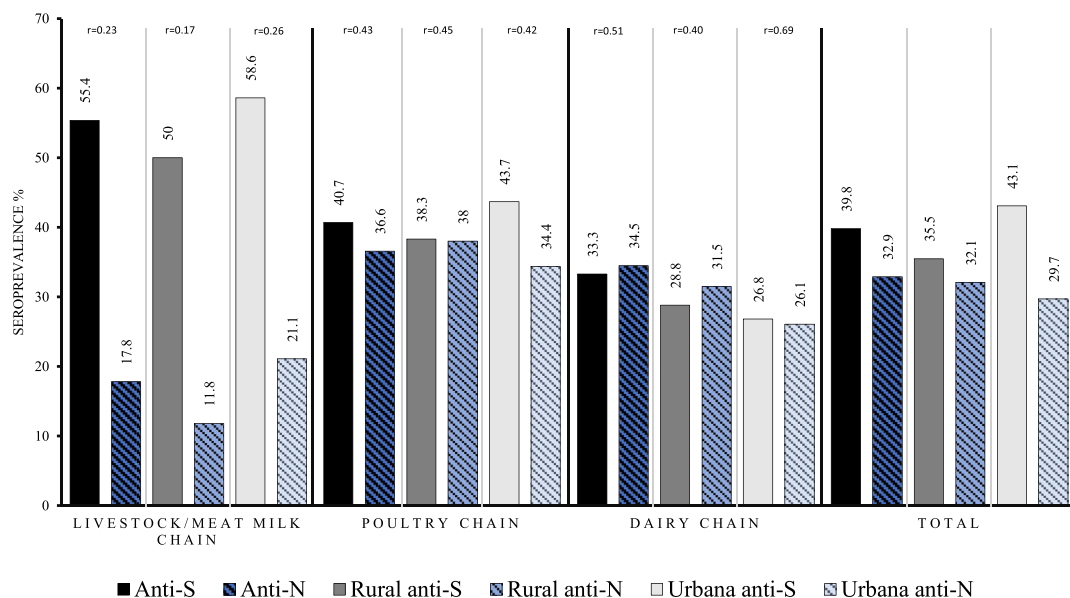


FIG. 1. Seroprevalence of antibodies against S and N protein of SARS CoV-2 in people linked to food production chains in Colombia. The value of the Pearson correlation coefficient (*r*) between the tests in each community is presented.

TABLE 3. Association between seropositivity to SARS-CoV-2 (COV2T test) and sociodemographic and health conditions in urban and rural workers linked to food-production chains in Colombia

	Urban			P ^a	Rural		
	COV2T test		P ^a		COV2T test		P ^a
	Negative n (%)	Positive n (%)			Negative n (%)	Positive n (%)	
Educational level							
Elementary school or less	218 (57.4)	162 (42.6)	0.459	210 (60.9)	135 (39.1)	<0.001	
Middle school or more	33 (52.4)	30 (47.6)		128 (75.3)	42 (24.7)		
Contact with COVID-positive people							
No	221 (59.9)	148 (40.1)	0.011	349 (64.6)	191 (35.4)	0.128	
Yes	33 (44.0)	42 (56.0)		17 (51.5)	16 (48.5)		
Inter-municipal travel							
No	220 (54.7)	182 (45.3)	0.017	349 (63.7)	199 (36.3)	0.292	
Yes	33 (73.3)	12 (26.7)		11 (52.4)	10 (47.6)		
Bus use as transportation							
No	230 (56.4)	178 (43.6)	0.542	325 (64.2)	181 (35.8)	0.296	
Yes	21 (61.8)	13 (38.2)		32 (57.1)	24 (42.9)		
Live with health workers							
No	236 (56.3)	183 (43.7)	0.546	364 (63.6)	208 (36.4)	0.866	
Yes	18 (62.1)	11 (37.9)		3 (60.0)	2 (40.0)		
Symptoms of COVID-19							
No	197 (55.2)	160 (44.8)	0.433	331 (65.0)	178 (35.0)	0.572	
Yes	48 (60.0)	32 (40.0)		45 (61.6)	28 (38.4)		
Number of cohabitants							
≤4	205 (59.2)	141 (40.8)	0.044	259 (65.7)	135 (34.3)	0.408	
>4	50 (48.1)	54 (51.9)		143 (62.4)	86 (37.6)		
Age (years)							
≤50	62 (61.4)	39 (38.6)	0.277	107 (71.8)	42 (28.2)	0.033	
>50	193 (55.3)	156 (44.7)		295 (62.2)	179 (37.8)		

^aChi Square test was performed. Bold values, values statistically significant.

presence; a historically weak health system; and non-compliance with regulations due to a lack of education or lack of resources. Regardless of the causes of the high seroprevalence, our findings indicate a large number of undiagnosed COVID-19 cases, either due to lack of access to diagnostic tests or because many cases were asymptomatic.

Seventy percent of seropositive cases did not report COVID-19 symptoms in the previous six months, which is a higher proportion than Oran and Topol reported for this disease (40%–45%) [26]. Asymptomatic infection could explain the rapid and increasing SARS-CoV-2 seroprevalence and why it is higher than the cumulative incidence based on PCR testing

TABLE 4. Association between seropositivity to SARS-CoV-2 (COV2T test) and sociodemographic and health conditions according food-production chain in Colombia

	Dairy chain			Livestock/meat and milk chain			Poultry chain		
	COV2T test		P	COV2T test		P	COV2T test		P
	Negative n (%)	Positive n (%)		Negative n (%)	Positive n (%)		Negative n (%)	Positive n (%)	
Educational level									
Elementary school or less	167 (70.8)	69 (29.2)	0.002	72 (45.3)	87 (54.7)	0.935	195 (57.7)	143 (42.3)	0.133
Middle school or more	58 (89.2)	7 (10.8)		12 (44.4)	15 (55.6)		93 (65.0)	50 (35.0)	
Contact with COVID-positive people									
No	249 (72.6)	94 (27.4)	0.011	76 (46.9)	86 (53.1)	0.272	266 (62.1)	162 (37.9)	0.002
Yes	14 (73.7)	5 (26.3)		10 (35.7)	18 (64.3)		26 (41.9)	36 (58.1)	
Inter-municipal travel									
No	248 (72.3)	95 (27.7)	0.713	82 (44.6)	102 (55.4)	<0.001	260 (58.0)	188 (42.0)	0.141
Yes	13 (68.4)	6 (31.6)		3 (42.9)	4 (57.1)		28 (70.0)	12 (30.0)	
Bus use as transportation									
No	214 (72.8)	80 (27.2)	0.043	84 (44.7)	104 (55.3)	0.267	268 (60.1)	178 (39.9)	0.152
Yes	40 (71.4)	16 (28.6)		1 (100)	0 (0)		21 (48.8)	22 (51.2)	
Number of cohabitants									
≤4	163 (76.5)	50 (23.5)	<0.001	70 (43.2)	92 (56.8)	0.388	235 (63.5)	135 (36.5)	<0.001
>4	196 (60.3)	129 (39.7)		16 (51.6)	15 (48.4)		58 (46.8)	66 (53.2)	
Age (years)									
≤50	138 (70.1)	59 (29.9)	0.214	13 (38.2)	21 (61.8)	0.414	62 (63.9)	35 (36.1)	0.303
>50	221 (64.8)	120 (35.2)		73 (45.9)	86 (54.1)		231 (58.2)	166 (41.8)	

in many countries [27–29]. At the end of 2021, the confirmed cases in Colombia by PCR were approximately 10% of the population, much lower than the seroprevalence reported in this and other seroepidemiological studies in the country [9–11]. This could be explained by the high percentage of asymptomatic patients who are not tested for confirmatory molecular tests. Therefore, close contact tracing and serological follow-up are fundamental tools in the public health surveillance programs in the region to interrupt the transmission of the SARS-CoV-2 virus.

On the other hand, estimation of the prevalence of prior infection from serosurveillance studies depends on the sensitivity of the test, the kinetics of antibody decay, and the type of antigen used. Follow-up studies have shown that antibodies against SARS-CoV-2 N protein decay faster with time than antibodies against S protein [30–32]. We find in the workers in the livestock/meat and milk chain, the highest antibody prevalence (55.4%) against S protein, and in turn, the lowest percentage of antibodies against N protein (17.8%) (Fig. 1). According to the kinetics of antibodies decaying against N protein, these workers were exposed early, as evidenced in the report of accumulated RT-PCR positive cases for the virus in this region of Colombia [6]. In the workers of the other food production chains, the percentages of antibodies against protein S and N were similar, indicating a more recent exposure to the virus. Taking into account that nucleocapsid antibody titers are undetectable approximately eight months after initial seroconversion [32], and that they are rapidly detected at the onset of infection, we consider that the N protein of SARS-CoV2 represents a good option to measure natural infection under the new conditions of the pandemic where most countries are already facing the third and fourth waves of the pandemic, with a considerable proportion of the population vaccinated, especially with protein S of the virus.

The major limitations of the present study were convenience sampling and the lack of infection confirmation by molecular tests. It is also important to note that many participants, especially in the dairy chain, refused to answer the socio-demographic survey, which probably skewed some interpretations in this community.

Conclusion

The high seroprevalence of SARS-COV-2 found in the present study based on food-producing communities in Colombia suggests an important interconnectivity between urban and rural areas, with the aggravating circumstance that most of those infected were asymptomatic, and that in rural areas the socio-demographic conditions were more precarious. It is important

to highlight that low schooling was only associated with the seropositivity of workers who lived in rural areas, so we reiterate the need to prioritize health education programs aimed at improving the knowledge of the population about COVID-19 vaccination to ensure compliance and application of prevention measures during the pandemic. Additionally, it is necessary to promote public health policies aimed at continuous epidemiological surveillance with different diagnostic tests.

Conflict of interest

The authors declare that they have no conflict of interest.

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