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Seroepidemiology and risk factors for SARS-CoV-2 infection among household members of food processing and farm workers in North Carolina

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ABSTRACT

Background: Racial and ethnic minorities have borne a disproportionate burden from coronavirus disease 2019 (COVID-19). Certain essential occupations, including food processing and farm work, employ large numbers of Hispanic migrant workers and have been shown to carry an especially high risk of infection.

Methods: This observational cohort study measured the seroprevalence of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and assessed the risk factors for seropositivity among food processing and farm workers, and members of their households, in North Carolina, USA. Participants completed questionnaires, blood samples were collected, and an enzyme-linked immunosorbent assay was used to assess SARS-CoV-2 seropositivity. Univariate and multi-variate analyses were undertaken to identify risk factors associated with seropositivity, using generalized estimating equations to account for household clustering.

Findings: Among the 218 participants, 94.5% were Hispanic, and SARS-CoV-2 seropositivity was 50.0%. Most seropositive individuals did not report a history of illness compatible with COVID-19. Attending church, having a prior history of COVID-19, having a seropositive household member, and speaking Spanish as one's primary language were associated with SARS-CoV-2 seropositivity, while preventive behaviours were not.

Interpretation: These findings underscore the substantial burden of COVID-19 among a population of mostly Hispanic essential workers and their households in rural North Carolina. This study contributes to a large body of evidence showing that Hispanic Americans have suffered a disproportionate burden of COVID-19. This study also highlights the epidemiologic importance of viral transmission within the household.

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Introduction

Early epidemiological studies indicated that certain essential occupations carried high risk for severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) exposure or outbreaks [1]. In both North Carolina (NC) and throughout the USA, meat packing plants, food processing facilities and commercial farms have served as foci for local outbreaks [2–4]. As employees of these facilities are considered essential workers, they are expected to continue working despite the elevated risk [5]. Rampant transmission of SARS-CoV-2 in these facilities has resulted in several high-profile plant closures [5–7]. Workers also infect their household members, contributing to transmission in surrounding communities [8]. In addition to their adverse effects on individuals' health, these focal outbreaks can overwhelm local medical and public health institutions, especially in underserved rural communities where these industries are concentrated. Outbreaks may also threaten the stability of the food supply due to plant closures and worker absenteeism from illness or exposure to SARS-CoV-2 [5,9].

Racial and ethnic minorities have suffered a disproportionate burden of coronavirus disease 2019 (COVID-19) morbidity and mortality [10], and similar trends have been seen among food industry workers. As of September 2022, 15% of COVID-19 cases in NC occurred among self-identified Hispanics, despite Hispanics accounting for only 10% of the state's population [11,12]. Since the beginning of the pandemic, the cumulative incidence of COVID-19 has been almost 20% higher among African-Americans and over 50% higher among Hispanics compared with non-Hispanic Whites, but this disparity was especially pronounced during the first 3 months of the pandemic, when the incidence of COVID-19 was as much as 10 times higher among Hispanics compared with non-Hispanic Whites [11]. Among agricultural workers in the USA, 87% of known cases of COVID-19 have occurred in racial or ethnic minorities, particularly among Hispanic individuals [4]. Many of these workers are recent, sometimes undocumented, immigrants to the USA, lack political and social capital to advocate for worker safety, and have limited access to health care [13].

Workers in the meat and poultry processing industry face an elevated risk of SARS-CoV-2 infection due to distinctive workplace conditions (i.e. close physical proximity between workers, cold temperatures, low humidity, metallic surfaces and poor ventilation) and community factors [14,15]. While outbreaks of COVID-19 in food processing facilities have been widely publicized, little is known about the transmission dynamics of the virus within the workers' households, and from there into the surrounding community. Since food processing and farm workers are likely to be considered essential in any future pandemics, and given the unique socio-economic features and healthcare infrastructure of the communities they inhabit, a focused approach is needed, not only to optimize workplace safety, but also to protect household members and surrounding communities. This observational cohort study of NC farming and food processing workers and their households was undertaken to investigate the seroprevalence of SARS-CoV-2 and risk factors for transmission in this population. The aims were to characterize the epidemiology of SARS-CoV-2 infections among NC farming and food processing workers and their household members; and to identify demographic, workplace, community and medical risk factors for past SARS-CoV-2 infection among the study population. NC is home to the second largest food processing industry in the USA [16]; as such, it provides an excellent setting to investigate whether and how the presence of this industry affects the epidemiology of COVID-19 in its vicinity, as well as the effects of the pandemic on this unique population of workers and their communities. Previously, the authors reported on workplace risk factors for SARS-CoV-2 infection in food processing and farm workers [15]. This paper presents data on the seroprevalence of SARS-CoV-2 and risk factors for seropositivity among these predominantly Hispanic workers' households, who were exceptionally highly affected in the early stages of the COVID-19 pandemic.

Materials and methods

Study setting

This study was conducted in central NC in an area with a large number of workers employed in food processing. Recruitment of study participants was conducted between September and December 2020. SARS-CoV-2 vaccines were not available to the general public during this period.

Study population

Adults aged ≥18 years who had worked for at least 2 weeks in a meat packing plant, food processing facility or commercial farm from 1 February 2020, and resided in NC were recruited and classified as index workers. Household members of index workers aged ≥12 months were invited to participate. Children aged <12 months were excluded because maternal antibodies transferred transplacentally could confound serological results. All adult participants provided written informed consent. A parent or legal guardian provided written parental permission for all children aged <18 years, and children aged 7–17 years provided written assent.

Data and specimen collection

Index participants were recruited by contacting potential food processing workers who received medical care at Piedmont Health Services, a federally qualified health centre with 10 community health centres in central NC, as well as public advertisement through flyers, social media campaigns and local community organizations. Study visits took place at the study office or under a tent outside the participants' residence. Participants attended an enrolment visit consisting of a questionnaire to collect data on demographic characteristics, medical history, household characteristics and preventive behaviours. Three biological samples (blood, saliva and nasal turbinate swab) were collected. Participants were invited to complete monthly follow-up visits until the end of December 2020 (maximum 4 months) when the same specimens were collected and a short follow-up questionnaire was administered to update exposure and clinical data. Participants were screened for COVID-19 symptoms or exposures with weekly telephone calls, and, if necessary, were referred to local clinics for free diagnostic testing. All study materials were available in English and Spanish, and were administered by bilingual study personnel. This study followed guidelines laid out in the STROBE statement [17].

Sample processing

Whole blood samples were collected by venepuncture or finger prick in standard EDTA tubes. The whole blood samples were centrifuged within 24 h of collection at 1600 *g* for 30 min to separate plasma. Prior to serological testing, all plasma specimens were heat-inactivated at 56°C for 30 min, mixing the sample by inverting the tube every 5 min. The inactivated samples were centrifuged at 1500 *g* for 10 min, and the supernatant was aliquoted and stored at -80°C until further testing.

Serological studies

Past infection with SARS-CoV-2 was measured using a total immunoglobulin (Ig) and IgM SARS-CoV-2 receptor binding domain (RBD) enzyme-linked immunosorbent assay (ELISA) that does not react with common endemic human coronaviruses, as described previously [18]. The spike protein N-terminal domain (NTD) antigen (16–305 amino acids, Accession: P0DTC2.1) was cloned into the pαH mammalian expression vector, and purified using nickel-nitrilotriacetic acid agarose in the same manner. To summarize the ELISA in brief, 50 μL of spike RBD antigen at 4 μg/mL in Tris buffered saline (TBS) pH 7.4 was coated in the

Table 1
Household characteristics associated with risk of having at least one seropositive household member.

At least one seropositive member	Yes = 49	No = 40	
Child <18 years present in household (*n*, %)	36/49 (73%)	24/40 (60%)	0.18[d]
Crowding (median, IQR)	**1.67 (1.25–2.00)**	**1.33 (1.00–1.67)**	**0.04**[b]
Household size (mean ± SD)	4.45 ± 1.99	3.89 ± 1.93	0.20[c]
Index worker occupation (*n*, %)			0.11[a]
Meat packing	25/49 (51%)	11/38 (29%)	
Food processing	1/49 (2%)	4/38 (11%)	
Farming	19/49 (39%)	18/38 (47%)	
Multiple workers in the house	4/49 (8%)	5/38 (13%)	
Home type (*n*, %)			0.61[a]
Single family home	16/49 (33%)	9/38 (24%)	
Apartment	2/49 (4%)	0/38 (0%)	
Duplex/townhouse	1/49 (2%)	1/38 (3%)	
Trailer	29/49 (59%)	26/38 (68%)	
Dormitory	1/49 (2%)	2/38 (5%)	

IQR, interquartile range; SD, standard deviation.
[a] Fisher's exact test.
[b] Wilcoxon rank sum test.
[c] Student's *t*-test.
[d] Chi-squared test.

96-well high-binding microtitre plate (Greiner Bio-One cat # 655061) for 1 h at 37°C. The plate was washed three times with 200 μL of wash buffer (TBS containing 0.2% Tween 20), and blocked with 100 μL of blocking solution (3% milk in TBS containing 0.05% Tween 20) for 1 h at 37°C. The blocking solution was removed, and 50 μL of serum sample at 1:20 or indicated dilutions in blocking buffer was added for 1 h at 37°C. The plate was washed in the wash buffer, and 50 μL of alkaline-phosphatase-conjugated secondary goat anti-human secondary antibody at 1:2500 dilution was added for 1 h at 37°C. In order to measure total Ig, a mixture of anti-IgG (Sigma Cat # A9544), anti-IgA (Abcam Cat # AB97212) and anti-IgM (Sigma Cat # A3437) were added together. The plate was washed, 50 μL of p-Nitrophenyl phosphate substrate (SIGMA FAST, Cat No N2770) was added to the plate, and absorbance was measured at 405 nm using a plate reader (Biotek Epoh, Model # 3296573). Optical density (OD) was measured with a VICTOR Nivo multi-mode plate reader (PerkinElmer, Waltham, MA, USA) 3, 5, 7, 9 and 11 min after the substrate was added. Samples were tested in duplicate, and duplicate values with variance >25% and/or one value above the assay cut-off were repeated.

Seventeen participants were unable to provide blood by venepuncture, so capillary blood was collected using a fingerprick method yielding <1 mL blood. These samples could not be separated by centrifugation and subsequently haemolysed during storage. As the plasma-based ELISA was inappropriate for these samples, antibody was detected using UNscience COVID-19 IgG/IgM rapid detection test strips; results were confirmed using a technique based on a previously described whole blood ELISA [19]. The rapid detection test results were confirmed and quantified using the same protocol parameters from the previous plasma-based ELISA through the substitution of whole blood for plasma and the addition of reconstituted whole blood controls for quality control. The reconstituted whole blood controls involved 55% plasma control and 45% erythrocytes to mimic blood proportions, which were further diluted in blocking buffer until the plasma control matched the sample dilutions on the plate. Whole blood ELISA titres of 1:20, 1:40 and 1:120 were compared with plasma-based ELISA OD readings using confirmed SARS-CoV-2 antibody negative and positive controls. The sensitivity and specificity of the 1:20 titre were obtained previously [20]. Sensitivity and specificity of the 1:40 and 1:120 titres were calculated [15].

Statistical analysis

Data were analysed using Stata Version 16 (IBM Corp., Armonk, NY, USA). Descriptive statistics were calculated, and Chi-squared test, Fisher's exact test, paired sample *t*-test and the rank sum test were used to assess for associations between individual characteristics or behaviours and SARS-CoV-2 seropositivity. Variables that were associated with SARS-CoV-2 seropositivity with $P<0.20$ on univariate analysis or which were deemed clinically or epidemiologically relevant were entered into a multi-variate model, using generalized estimating equations to account for clustering of cases within households. Variables were then removed in a backward stepwise manner until the most parsimonious model was achieved.

Ethics

This study was approved by the Institutional Review Board at the University of North Carolina at Chapel Hill (IRB 20-2032).

Results

In total, 224 participants in 90 households were enrolled between September and December 2020. Enrolment blood samples were available for 218 participants in 89 households, including 118 index workers and 100 household members. The median age of participants was 34.1 years (interquartile range 14.3–46.6), 32.1% were aged <18 years, 54.6% were female, 94.5% were Hispanic, and 74.8% spoke Spanish as their primary language. Index workers were more likely to be female ($P=0.04$) and Spanish speaking ($P<0.01$) than household members. Baseline seroprevalence of SARS-CoV-2 was 50% among both index workers (59/118) and household members (50/100). Sixty percent of seropositive participants reported no known history of COVID-19-compatible illness.

Forty (44.9%) of the 89 households had no seropositive cases. Forty-nine households (55.1%) had at least one seropositive case, and 35 of these households had 100% seropositivity among household members. Household crowding, defined as the number of individuals in the household divided by the number of bedrooms, was associated with higher risk of having at least one seropositive household member, while type of home, presence of children aged <18 years and occupation of the index worker were not (Table 1).

Among individual participants, attending church, having a prior history of COVID-19 (either confirmed by polymerase chain reaction or based on clinical diagnosis by a healthcare professional) and having a seropositive household member were significantly associated with SARS-CoV-2 seropositivity on both univariate and multivariate analyses (Tables 2 and 3). The presence of children in the

Table 2
Univariate analysis assessing for associations between preventive behaviours; demographic, clinical and household characteristics; and individual risk of severe acute respiratory syndrome coronavirus-2 seropositivity.

	Positive *n*=109 *n* (%)	Negative *n*=109 *n* (%)	*P*-value
Demographics			
Age (median, IQR)	33.9 (13.8–45.5)	34.8 (14.6–48.1)	0.32
Male sex	51/109 (47%)	48/109 (44%)	0.68
Hispanic ethnicity	104/109 (95%)	102/109 (94%)	0.77
Spanish speaker	85/109 (78%)	78/109 (72%)	0.27
Household member	50/109 (46%)	50/109 (46%)	1.00
Home type			0.20[a]
Single family	37/109 (34%)	27/107 (25%)	
Apartment	3/109 (3%)	0/107(0%)	
Duplex/townhouse	2/109 (2%)	1/107 (1%)	
Trailer	66/109 (61%)	77/107 (72%)	
Dorm	1/109 (1%)	2/107 (2%)	
School, travel and social activities (past 2 weeks)			
Travelling	10/109 (9%)	8/109 (7%)	0.67
Attending in-person class	22/32 (69%)	21/34 (62%)	0.55
Employed	60/80 (75%)	57/76 (75%)	1.00
Visiting healthcare facility	15/109 (14%)	14/106 (13%)	0.91
Visiting school or daycare	32/109 (29%)	30/106 (28%)	0.86
Visiting grocery store	78/109 (72%)	76/106 (72%)	0.98
Visiting mall or other shopping site	86/109 (79%)	80/106 (75%)	0.55
Visiting restaurant	31/109 (28%)	32/106 (30%)	0.78
Visiting another person's home	26/109 (24%)	37/106 (35%)	0.07
Visiting church	**10/109 (9%)**	**1/106 (1%)**	**<0.01**[a]
Socializing outside of home	44/108 (41%)	38/108 (35%)	0.40
Going to sporting event	0/109 (0%)	1/106 (1%)	0.49[a]
Social distancing measures and hygiene habits			
Staying home from school/work	20/108 (19%)	28/108 (26%)	0.19
Avoiding large groups	87/108 (81%)	86/108 (80%)	0.86
Avoiding high-risk people	84/108 (78%)	80/108 (74%)	0.52
Staying 6 feet away from other people	104/108 (96%)	98/108 (91%)	0.10[a]
Hand washing	107/108 (99%)	104/109 (95%)	0.21[a]
Using hand sanitizer	95/108 (88%)	96/109 (88%)	0.98
Wearing a mask	103/108 (95%)	104/109 (95%)	0.99[a]
Wearing eye protection	19/108 (18%)	11/109 (10%)	0.11
Cleaning personal items	60/108 (56%)	50/109 (46%)	0.15
Cleaning home more often	80/108 (74%)	74/109 (68%)	0.32
Prior COVID-19 diagnoses and exposures			
Prior COVID-19 diagnosis	**44/109 (40%)**	**6/109 (6%)**	**<0.01**
Household member diagnosed with COVID-19	**58/108 (54%)**	**14/109 (13%)**	**<0.01**
Household member with positive serology	**89/109 (82%)**	**20/109 (18%)**	**<0.01**
Exposed to suspected or confirmed COVID-19 case	7/109 (6%)	5/108 (5%)	0.56[a]
Co-morbidities			
Diabetes	12/108 (11%)	19/109 (17%)	0.18
Hypertension	14/108 (13%)	14/109 (13%)	0.98
Asthma	2/109 (2%)	5/109 (5%)	0.25[a]
Any underlying condition	30/109 (28%)	37/109 (34%)	0.30
Ever been a smoker	15/109 (14%)	12/109 (11%)	0.54
Alcohol use	19/109 (17%)	17/109 (16%)	0.72
Any substance use	3/104 (3%)	1/107 (1%)	0.36[a]
Household characteristics			
Child <18 years present in household	**93/109 (85%)**	**79/107 (74%)**	**0.04**
Crowding (median, IQR)	**1.67 (1.50–2.00)**	**1.50 (1.00–2.00)**	**<0.01**[b]
Household size (mean ± SD)	**4.94 ± 1.75**	**4.43 ± 1.71**	**0.03**[c]

IQR, interquartile range; COVID-19, coronavirus disease 2019.
[a] Fisher's exact test.
[b] Wilcoxon rank sum.
[c] Student's *t*-test.Chi-squared test was used unless otherwise noted.

household, as well as household crowding and higher absolute number of people in the household, were significantly associated with seropositivity on univariate analysis ($P<0.05$) but not on multi-variate analysis. Furthermore, the multi-variate analysis revealed that speaking Spanish as a primary language was associated with a higher rate of seropositivity. Sex, race, preventive behaviours (e.g. mask wearing, hand washing), being employed, attending school, travelling, visiting public spaces other than church, co-morbidities, smoking, alcohol and substance use were not associated with SARS-CoV-2 serostatus.

Discussion

A cohort of food processing and farm workers from NC and their household members were analysed in order to determine SARS-CoV-2 seroprevalence and identify epidemiological risk factors for SARS-CoV-2 infection among this population in the early stages of the COVID-19 pandemic. As of the end of December 2020, when this study ended, 547,452 cases of COVID-19 had been reported in NC, a cumulative incidence of 5.2% [12,21]. This study found that exactly half of the cohort had antibodies to SARS-CoV-2, which was much higher seroprevalence than was

Table 3
Multi-variate model showing behaviours, demographic and clinical characteristics, and exposures associated with severe acute respiratory syndrome coronavirus-2 seropositivity.

	Positive *n*=109 *n* (%) or median (IQR)	Negative *n*=109 *n* (%) or median (IQR)	aOR (95% CI)
Age	33.9 (13.8–45.5)	34.8 (14.6–48.1)	1.00 (0.99–1.00)
Male sex	51/109 (47%)	48/109 (44%)	1.02 (0.93–1.12)
Latinx ethnicity	104/109 (95%)	102/109 (94%)	0.87 (0.69–1.10)
Spanish speakers	**85/109 (78%)**	**78/109 (72%)**	**1.20 (1.05–1.38)**
Visiting church	**10/109 (9%)**	**1/106 (1%)**	**1.33 (1.07–1.66)**
Prior COVID-19 diagnosis	**44/109 (40%)**	**6/109 (6%)**	**1.42 (1.26–1.59)**
Household member with positive serology	**89/109 (82%)**	**20/109 (18%)**	**1.75 (1.59–1.94)**

aOR, adjusted odds ratio; CI, confidence interval; IQR, interquartile range; COVID-19, coronavirus disease 2019. Generalized estimating equations were used to account for clustering of cases within households.

estimated by both reported cases in NC and large serosurveys at that time, which calculated that, by December 2020, the seroprevalence of SARS-CoV-2 had reached almost 14% among the NC general population and 33.5% among NC Hispanics [22], and 11.4% in the general population and almost 20% among Hispanics nationwide [23]. This highlights the disproportionate vulnerability to infection of the study population in the first months of the pandemic.

The majority of seropositive participants had no reported history of COVID-19. Consistent with numerous past studies, this reflects the high rate of asymptomatic and pauci-symptomatic infections, and may also reflect the scarcity of testing during the first months of the pandemic [24,25]. While the availability of SARS-CoV-2 tests was generally limited in the early stages of the pandemic, data collected in NC up to June 2020 revealed much higher test positivity rates among Hispanics and African-Americans compared with non-Hispanic Whites, as well as among rural populations compared with urban communities, reflecting even poorer testing availability among these populations, and highlighting another significant disparity [25].

Personal and household history of COVID-19 were strongly associated with SARS-CoV-2 seropositivity, as expected. The role of preventive habits and other behavioural risk factors is less clear. Visiting church was associated with seropositivity, but visiting other public spaces such as malls, restaurants, grocery stores and other people's homes was not. Social distancing and hygiene habits (hand washing, mask wearing, etc.) were not associated with seropositivity. Taken together, these results highlight the importance of transmission within the household. People are unlikely to adhere to mask wearing and physical distancing within their own home, thus they are more likely to be infected by a contagious household member regardless of preventive behaviours practiced outside the home. Furthermore, even if sick individuals use masks or isolate from household members, pre-symptomatic or asymptomatic individuals may still transmit infection. Multiple studies from all over the world have shown high SARS-CoV-2 household secondary attack rates, and how socio-economic factors can affect viral transmission within the household [26–32]. Notably, as this study was conducted in Autumn 2020, a significant number of participants were probably infected during the first wave of the pandemic (March–July 2020), when the incidence of COVID-19 was much higher among Hispanics than other ethnic groups in NC [11]. At this time, mask wearing was not yet widespread, or participants may have been wearing masks incorrectly.

The observed association in this mostly Hispanic population between speaking Spanish as a primary language and SARS-CoV-2 seropositivity was unexpected and noteworthy. It is posited that language in this case is a proxy for time spent in the USA, with more recent immigrants being more likely to speak primarily Spanish. As such, the observed association may reflect systemic vulnerabilities experienced by people who are more recent immigrants to the USA. Two Canadian studies have suggested that, during the first year of the pandemic, recent immigrants were more likely to test positive for SARS-CoV-2 and experienced disproportionately high mortality from COVID-19 [33,34].

One limitation of this study was the use of a convenience sample which may not have reflected the true seroprevalence of SARS-CoV-2 in this population accurately. In addition, this study could not include individuals who died of COVID-19, which possibly introduces selection bias. Behavioural data were collected by self-report, which is susceptible to recall and social desirability bias. Furthermore, current behaviour may not reflect behaviours performed earlier in the COVID-19 pandemic accurately, as public health guidelines evolved rapidly. These considerations may account for some of the unexpected results, such as the lack of association between preventive behaviours and seropositivity. Last but not least, 17 participants were not able to provide a blood sample by venepuncture, and capillary blood had to be obtained instead. These samples had to be processed by a different method, which could have introduced discrepancy in some of these results, despite the measures taken to minimize this risk.

Despite its limitations, this study does shed light on the seroepidemiology of SARS-CoV-2 among a particularly vulnerable and often overlooked group of largely Hispanic essential workers and their communities in the USA. An analysis of publicly available data suggests that meat processing plants facilitate the spread of SARS-CoV-2 into the surrounding communities. It has been estimated that up to 8% of all cases of COVID-19 and 4% of COVID-19-related deaths in the USA prior to 21 July 2020 were associated with proximity to a meat processing plant, and most of these related to community transmission around the plants [8]. Moreover, there is a growing body of evidence demonstrating the disproportionate burden of disease experienced by US Hispanics due to COVID-19, likely due to a variety of social determinants of health such as lower socio-economic status, living in multi-generational households, lack of access to health care, language barrier and essential worker status [35]. The present study provides further evidence of this disparity, and suggests that the intersection between these social determinants of health and the food industry may have exacerbated the impact of the pandemic on the Hispanic community in rural NC.

In summary, this study identified a remarkably high seroprevalence of SARS-CoV-2 in the early stages of the pandemic among a predominantly Hispanic population of NC food processing and farm workers and their household members. These findings highlight the burden of the COVID-19 pandemic in this vulnerable population, and the role that food processing may play in community spread, as well as the importance of viral transmission within the household.

Conflict of interest statement

None declared.

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