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SHORT COMMUNICATION

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A cross-sectional community-based observational study of asymptomatic SARS-CoV-2 prevalence in the greater Indianapolis area

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Abstract

The Asymptomatic novel CORonavirus iNfection (ACORN) study was designed to investigate the prevalence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in the asymptomatic adult population of the Indianapolis metropolitan area, to follow individuals testing positive for the development of symptoms, and to understand duration of positive test results. ACORN is a cross-sectional community-based observational study of adult residents presenting asymptomatic for COVID-like illness, defined as the self-reported absence of the following three symptoms in the last 7 days: fever (≥100°F), new-onset or worsening cough, and new-onset or worsening shortness of breath. SARS-CoV-2 infection was determined by real-time reverse transcription-polymerase chain reaction in nasopharyngeal swab samples. SARS-CoV-2 infection prevalence was expressed as a point estimate with 95% confidence interval (CI). Test results are reported for 2953 participants who enrolled and underwent nasopharyngeal swab testing between 7 April 2020 and 16 May 2020. Among tested participants, 91 (3.1%; 95% CI: 2.5%-3.7%) were positive for SARS-CoV-2. Overall, baseline characteristics, medical history, and infection risk factors were comparable between SARS-CoV-2 positive and negative participants. Within the ongoing 14-day follow-up period for positive participants, 58 (71.6%) of 81 assessed participants remained asymptomatic while others (n = 23, 28.4%) reported one or more symptoms. Indiana had "Stay-at-Home" orders in place during nearly the entire test period reported here, yet 3.1% of asymptomatic participants tested positive for SARS-CoV-2. These results indicate screening questions had limited predictive utility for testing in an asymptomatic population and suggest broader testing strategies are needed. Importantly, these findings underscore that more research is needed to understand the viral transmission and the role asymptomatic and presymptomatic individuals play in this global pandemic.

KEYWORDS

coronavirus, epidemiology, pandemic

1 | INTRODUCTION

The World Health Organization first declared a pandemic due to the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) on 11 March 2020.[1] As of 18 May 2020, over 4.7 million people have confirmed SARS-CoV-2 infection worldwide and over 315 000 deaths.[2] Important for understanding the nature of the pandemic, and efforts for slowing its spread, is to assess the number of persons affected across the disease spectrum.[3] Repeated studies have confirmed a high proportion of asymptomatic cases, with current estimates indicating between 18% and 50% of SARS-CoV-2 cases in local or contained outbreaks are asymptomatic.[4-7] Most testing efforts for SARS-CoV-2 infection in the early months of the pandemic have focused on symptomatic people, persons at high risk due to occupational exposure, or persons at high risk for adverse outcomes. Further, the transmission of the virus has been documented from asymptomatic persons.[4,7] As such, studies to understand the extent of SARS-CoV-2 infection among asymptomatic populations are of specific interest.

The Asymptomatic novel CORonavirus iNfection (ACORN) study was designed to investigate the prevalence of SARS-CoV-2 infection in the asymptomatic adult population of the Indianapolis (Indiana) metropolitan area, and to follow individuals testing positive for the development of symptoms and to understand the duration of positive test results. Prevalence data from the ACORN study are presented here, along with interim follow-up data.

2 | METHODS

ACORN is a cross-sectional community-based observational study of adult residents presenting asymptomatic for COVID-like illness. Asymptomatic was defined as the self-reported absence of the following three symptoms within the last 7 days: fever (≥100 °F), new-onset or worsening cough, and new-onset or worsening of shortness of breath. The primary outcome of the study is SARS-CoV-2 infection prevalence. Enrollment began on 6 April 2020 and continued through 16 May 2020. A nested longitudinal study for participants who test positive for SARS-CoV-2 is ongoing to investigate symptom development at approximately 2 weeks postindex testing. Further, participants who test positive are invited to return for repeat testing at approximately 2-week intervals until the nasopharyngeal swab result is negative for SARS-CoV-2 infection (for a maximum of three additional tests). The study protocol was approved by an external Institutional review board (IRB) (WIRB-Copernicus) and performed in compliance with relevant regulations and in accordance with the ethical standards of the Declaration of Helsinki. All participants provided informed consent.

2.1 | Study enrollment

Study participants were recruited through media announcements and a publicly accessible website with study documents and information. The study website provided information about the inclusion and exclusion criteria, and directed interested persons to call to enroll. Collection of verbal and electronic informed consent, eligibility determination, completion of a brief questionnaire, and scheduling of testing were completed over the phone. Nasopharyngeal swab collection was scheduled to occur within 48 hours of telephone enrollment.

2.2 | Inclusion and exclusion criteria

Eligible participants were ≥18 years of age, residents of the Indianapolis Metropolitan area (resident of Marion, Hamilton, Hendricks, Johnson, Madison, Hancock, Morgan, Boone, Shelby, Putnam, or Brown county), have access to a personal vehicle to drive to Lilly Corporate Center for SARS-CoV-2 testing, and have access to an internet-connected electronic device. Individuals were excluded if they had previously tested positive for SARS-CoV-2, had previously enrolled in the study, or reported a fever (≥100 °F) or physical symptoms of a fever (in absence of a thermometer), new-onset or worsening cough, new-onset or worsening of shortness of breath in the past 7 days.

2.3 | Sample collection and testing

Samples were collected in-person (within 48 hours of screening) at a drive-through testing facility from 7 April 2020 to 16 May 2020. Samples were collected by trained personnel according to centers for disease control and prevention (CDC) recommended methods for nasal pharyngeal swabs and included swabbing of each nostril with same swab.[8] SARS-CoV-2 infection was determined by real-time reverse transcription-polymerase chain reaction (RT-PCR) in nasopharyngeal swab samples.[9] The SARS-CoV-2 qualitative RT-PCR test was analytically validated using CDC primer and probe set(s) in the Clinical Diagnostics Laboratory, Eli Lilly and Company, that is a Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a certified high-complexity laboratory. The validation was designed to meet the Food and Drug Administration's (FDA) Emergency Use Authorization for SARS-CoV-2 PCR testing and submitted to both the FDA and the Indiana State Department of Health for review. The assay validation demonstrated sensitivity and specificity of 100% and a limit of detection of 1000 copies/mL.

Test results were reported to the Indiana State Health Department and provided to participants via a password-protected website in approximately 1 to 3 days. Participants who test positive were also contacted within 3 days of results becoming available to confirm receipt of results, provide information about self-isolation and close contact notification per state recommendations for persons testing positive, and invited to participate in repeat testing every 2 weeks, or until the test is negative. Further, participants who test positive are re-contacted at approximately 14 days posttesting to query for symptom development. No follow-up was required for SARS-CoV-2

TABLE 1 Participant demographics, basic characteristics, baseline symptoms, and SARS-CoV-2 exposure risk factors. Results are presented as n (%) unless noted otherwise.

	Positive N = 91	Negative N = 2862	Overall N = 2953
Age, mean (SD), y	48.1 (16.3)	49.6 (15.4)	49.6 (15.5)
18 to <30	18 (19.8)	351 (12.3)	369 (12.5)
30 to <40	13 (14.3)	503 (17.6)	516 (17.5)
40 to <50	12 (13.2)	481 (16.8)	493 (16.7)
50 to <60	20 (22.0)	650 (22.7)	670 (22.7)
60 to <70	21 (23.1)	608 (21.2)	629 (21.3)
≥70	7 (7.7)	269 (9.4)	276 (9.3)
Gender			
Female	58 (63.7)	1682 (58.8)	1740 (58.9)
Male	33 (36.3)	1180 (41.2)	1213 (41.1)
Race			
White	79 (86.8)	2572 (89.9)	2651 (89.8)
Black	7 (7.7)	158 (5.5)	165 (5.6)
Asian	1 (1.1)	59 (2.1)	60 (2.0)
American Indian or Alaska Native	0 (0.0)	4 (0.1)	4 (0.1)
Native Hawaiian or Other Pacific Islander	0 (0.0)	1 (0.0)	1 (0.0)
Other	4 (4.4)	68 (2.4)	72 (2.4)
Ethnicity			
Hispanic or Latino	3 (3.3)	93 (3.2)	96 (3.3)
Not Hispanic or Latino	88 (96.7)	2769 (96.8)	2857 (96.7)
BMI			
<25	33 (36.3)	1031 (36.0)	1064 (36.0)
25 to <30	31 (34.1)	1050 (36.7)	1081 (36.6)
≥30	27 (29.7)	781 (27.3)	808 (27.4)
Number of people in household[a], mean (SD)	1.6 (1.3)	1.6 (1.3)	1.6 (1.3)
Overall general health			
Excellent	47 (51.6)	1287 (45.0)	1334 (45.2)
Good	40 (44.0)	1457 (50.9)	1497 (50.7)
Fair	4 (4.4)	116 (4.1)	120 (4.1)
Poor	0 (0.0)	2 (0.1)	2 (0.1)
Preexisting conditions			
Participants with ≥1 condition	43 (47.3)	1435 (50.1)	1478 (50.1)
Hypertension	18 (19.8)	579 (20.2)	597 (20.2)
Cardiovascular disease	2 (2.2)	138 (4.8)	140 (4.7)
Asthma	5 (5.5)	257 (9.0)	262 (8.9)
Chronic lung disease[b]	2 (2.2)	33 (1.2)	35 (1.2)
Diabetes mellitus	2 (2.2)	131 (4.6)	133 (4.5)
Chronic renal disease	0 (0.0)	14 (0.5)	14 (0.5)
Chronic liver disease	1 (1.1)	12 (0.4)	13 (0.4)
Immunocompromised condition	6 (6.6)	151 (5.3)	157 (5.3)
Neurological/neurodevelopmental disorder	2 (2.2)	51 (1.8)	53 (1.8)
Currently pregnant[c]	0 (0.0)	21 (1.2)	21 (1.2)
Current smoker	1 (1.1)	114 (4.0)	115 (3.9)
Former smoker	23 (25.3)	691 (24.1)	714 (24.2)
Infection risk factors[d]			
Participants with ≥1 risk factor	49 (53.8)	1417 (49.5)	1466 (49.6)
Any travel outside the state of Indiana[e]	9 (9.9)	138 (4.8)	147 (5.0)
Contact with any person with a confirmed COVID-19 infection[e,f]	11 (12.1)	256 (8.9)	267 (9.0)

TABLE 1 (Continued)

	Positive N = 91	Negative N = 2862	Overall N = 2953
Contact with any person with suspected COVID-19 or flu-like symptoms[e,g]	5 (5.5)	211 (7.4)	216 (7.3)
Current travel to a workplace other than the participant's home[h]	24 (26.4)	744 (26.0)	768 (26.0)
Any household member traveling to a workplace outside the home	21 (23.1)	850 (29.7)	871 (29.5)
Baseline symptoms			
Participants with ≥1 symptom	13 (14.3)	479 (16.7)	492 (16.7)
Chills	0 (0.0)	8 (0.3)	8 (0.3)
Fatigue or muscle aches	2 (2.2)	82 (2.9)	84 (2.8)
Sore throat	1 (1.1)	62 (2.2)	63 (2.1)
Headache	7 (7.7)	322 (11.3)	329 (11.1)
Gastrointestinal symptoms[i]	4 (4.4)	91 (3.2)	95 (3.2)
Loss of smell or taste	3 (3.3)	21 (0.7)	24 (0.8)

Abbreviations: BMI, body mass index (kg/m^2); CI, confidence interval; COVID-19, Coronavirus Disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SD, standard deviation.

[a]Excluding self.

[b]Emphysema or Chronic Obstructive Pulmonary Disease.

[c]Percentage calculated relative to number of female participants.

[d]Percentages for infection risk factors may not add to 100% because participants were allowed to select multiple risk factors.

[e]Within the past 14 days.

[f]Among participants reporting contact with any person with a confirmed COVID-19 infection (n = 267), 60 overall reported it was a member of the participant's household (3 positive, 57 negative).

[g]Among participants reporting contact with any person with suspected COVID-19 or flu-like symptoms (n = 216), 59 overall reported it was a member of the participant's household (2 positive, 57 negative).

[h]Among participants traveling to a workplace other than the participant's home (n = 768), 187 were healthcare professionals (4 positive, 183 negative), 8 were first responders (0 positive, 8 negative), 398 were essential workplace employees (13 positive, 385 negative), and 175 were classified other (7 positive, 168 negative).

[i]Nausea, vomiting, or diarrhea.

negative participants, but all participants have secure online access to their results.

2.4 | Statistical analysis

SARS-CoV-2 infection prevalence was expressed as a point estimate with 95% confidence interval (CI) using a Wald interval (normal approximation). A sample size of 3000 was prespecified to estimate an assumed true SARS-CoV-2 prevalence of 1% with a 0.36% margin of error for a 95% CI. Baseline characteristics, medical history, and infection risk factor results are descriptive with no statistical inferences made. Reported results were not adjusted for confounding effects, bias, or multiplicity. Participants with missing questionnaire data were excluded from analyses dependent upon the missing data.

3 | RESULTS

ACORN enrolled participants 6 April 2020 to 16 May 2020. During this period, 3120 participants enrolled in the study and scheduled for a swabbing appointment. Among enrolled participants, 167 did not have evaluable samples, either due to withdrawal of consent after the telephone enrollment and before arriving for testing or no-show for the swabbing appointment. SARS-CoV-2 test results were reported for 2953 participants.

3.1 | Prevalence results

Among tested participants, 91 (3.1%; 95% CI: 2.5%-3.7%) tested positive for SARS-CoV-2. Demographics and health characteristics are provided in Table 1. Baseline characteristics, medical history, and overall infection risk factors were generally consistent between SARS-CoV-2 positive and negative participants (Table 1). However, prevalence of SARS-Cov-2 infections appeared to be numerically higher for participants in the age group of 18 to less than 30 years of age (4.9% tested positive) (Table 1). The majority of infection risk factors were comparable between groups, however, among SARS-CoV-2 positive participants, 9 (9.9%) reported travel outside the state of Indiana and 11 (12.1%) reported contact with a confirmed SARS-CoV-2 infection compared to 138 (4.8%) and 256 (8.9%), respectively, among SARS-CoV-2 negative participants (Table 1). At enrollment, participants were required to be free of new-onset or

worsening fever, cough, and shortness of breath. However, additional nonspecific symptoms were collected. Among all participants, 492 (16.7%) reported at least one symptom at enrollment with headaches being most frequently reported (11.1%) (Table 1). Proportion of positive and negative participants reporting these nonspecific symptoms were generally consistent.

3.2 | Follow-up among participants positive for SARS-CoV-2

At the time of this analysis, 81 participants had completed a follow-up interview at 14 days to query for development of symptoms. Among these participants, 58 (71.6%) remained asymptomatic and 23 (28.4%) reported at least one symptom during the 14-day follow-up period after the test date (Table 2). The most frequently reported symptoms were headaches (12.3%), fatigue and/or muscle aches (11.1%), and shortness of breath (8.6%). Additionally, 65 of the 81 follow-up participants submitted repeat nasopharyngeal swabs for RT-PCR testing at the time of this report. Of these, 16 (24.6%) remained positive at approximately 14 days postindex test date. At the time these analyses were conducted, follow-up was ongoing for the remaining participants who tested positive.

4 | DISCUSSION

Understanding the extent of SARS-CoV-2 infection among asymptomatic people in different metropolitan areas is of interest due to reports of asymptomatic transmission and lacking data to inform the prevalence of infection in a nonselected sample (as most testing to date has been focused on symptomatic or high-risk groups). A recent serosurvey in Santa Clara County (California) estimated that SARS-CoV-2 prevalence was 2.8% (95% CI: 1.3%-4.7%), approximately 55-fold higher than reported in the county during the investigated time period, although it was not designed to evaluate asymptomatic prevalence and a serosurvey in Los Angeles County (California) conducted approximately 2 weeks later estimated the prevalence in a random sample to be 4.65%. Despite Indiana having "Stay-at-Home" orders effective from 24 March 2020 to 4 May 2020 (11 May 2020 for residents of Marion County [including Indianapolis]), 3.1% of asymptomatic participants tested positive for SARS-CoV-2 in ACORN (between 7 April 2020 and 16 May 2020) based on RT-PCR. This is consistent with preliminary results from a study conducted by the Indiana State Department of Health and the Fairbanks School of Public Health that included symptomatic and asymptomatic individuals where the prevalence was approximately 2.8% statewide (1.7% with active infection determined by RT-PCR, and additional 1.1% testing positive for antibodies) and 4.2% within the Indiana Public Health Preparedness District 5, which includes 8 of the 11 counties in ACORN.[10] Of those with active infection, 45% reported no symptoms at all, underscoring this is a significant proportion of the impacted population.[10]

TABLE 2 Symptoms developed within approximately 14 days after SARS-CoV-2 diagnosis among participants who completed follow-up at the time of the interim analysis (N = 81)

Reported symptoms	n (%)
Subjects with ≥1 symptom	23 (28.4)
Fever (temperature ≥100 °F)	4 (4.9)
Chills	3 (3.7)
Fatigue and/or muscle aches	9 (11.1)
Sore throat	4 (4.9)
Cough	6 (7.4)
Shortness of breath	7 (8.6)
Headache	10 (12.3)
Gastrointestinal symptoms[a]	4 (4.9)
Loss of smell or taste	6 (7.4)

Abbreviation: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.
[a]Nausea, vomiting, or diarrhea.

Overall in the ACORN study, self-reported risk for exposure to the virus was consistent between SARS-CoV-2 positive and negative participants, indicating exposure is possible despite movement restrictions. These data also suggest that these screening questions did not clearly identify the "at-risk" individuals for focused asymptomatic screening. The identified prevalence of asymptomatic SARS-CoV-2 infection in the community from the ACORN study, the high proportion of asymptomatic cases previously identified among those who test positive,[4-7] and the lack of clear risk-based identifiers emphasize that testing and contact tracing focused solely on symptomology or risk-based assessment may be insufficient. Instead, data suggest that broader population screening and testing beyond symptomatic and high risk may be needed for identification of individuals infected with SARS-CoV-2.

Limitations of the study include the self-selected participation rather than a randomized sample. The self-selected nature of participation resulted in a study population not fully representative of the greater Indianapolis area. For example, 89.8% of ACORN participants in this interim analysis were White and 5.6% Black, whereas based on census data from the 11 counties participating in ACORN, 78% are White and 16% are Black.[11] The ACORN population may, therefore, underestimate the SARS-CoV-2 prevalence, as previous research reported nearly twice the prevalence among non-White residents of Indiana compared to White.[10] Additionally, eligibility determination (including absence of fever, cough, and shortness of breath) and questionnaire data were all self-reported. As such, there is a possibility of false reporting of symptoms status upon enrollment. Likewise, there is possible reporting bias of symptoms at follow-up among participants who tested positive, as knowledge of a positive test result could influence awareness and reporting of symptoms. Because a small proportion of participants did go on to develop and report symptoms at the 14-day follow-up, this study captured SARS-CoV-2 infections that were both asymptomatic and presymptomatic. Follow-up is ongoing

for remaining participants who tested positive and differentiation between asymptomatic and presymptomatic will be reported in final analyses. Finally, it is not known if participants with positive RT-PCR testing are infectious, or their index date of infection.

These results from ACORN indicate that 3.1% of a self-selected population of asymptomatic individuals were positive for SARS-CoV-2. Of those, most remained asymptomatic (72%) and a small percentage were presymptomatic (28%). The symptoms reported among the presymptomatic participants were variable and generally mild. These prevalence and interim follow-up data contribute to our understanding of the asymptomatic and presymptomatic populations with SARS-CoV-2 and may be useful for consideration as public health officials assess testing strategies, recommendations for movement restrictions, and for use in modeling transmission and the impact of the epidemic in metropolitan areas. These findings underscore that more research is needed to understand the viral transmission and the role asymptomatic and presymptomatic individuals play in this global pandemic.

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CONFLICT OF INTERESTS

All authors are employees and shareholders of Eli Lilly and Company.

AUTHOR CONTRIBUTIONS

All authors participated in the drafting and critical revision of the manuscript. All authors approved the final submitted version of the manuscript.

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