

Title

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

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Running Head: Asymptomatic SARS-CoV-2 Prevalence

ABSTRACT

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 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

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self-reported absence of the following 3-symptoms in the last 7-days: fever ($\geq 100^{\circ}\text{F}$), new onset or worsening cough, and new onset or worsening shortness of breath. SARS-CoV-2 infection was determined by RT-PCR in nasopharyngeal swab samples. SARS-CoV-2 infection prevalence was expressed as a point estimate with 95%-CI. Test results are reported for 2953 participants who enrolled and underwent nasopharyngeal swab testing between April 7, 2020 and May 16, 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 pre-symptomatic individuals play in this global pandemic.

KEY WORDS: Coronavirus, Epidemiology, Pandemic

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, transmission of 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.

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 3 symptoms within the last 7 days: fever ($\geq 100^{\circ}\text{F}$), 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 April 6, 2020 and continued through May 16, 2020. A nested longitudinal study for participants who test positive for SARS-CoV-2 is ongoing to investigate symptom development at approximately 2 weeks post-index 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 3 additional tests). The study protocol was approved by an external 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.

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.

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 ($\geq 100^{\circ}\text{F}$) 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.

Sample Collection and Testing

Samples were collected in-person (within 48 hours of screening) at a drive-through testing facility from April 7, 2020 to May 16, 2020. Samples were collected by trained personnel according to 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 FDA's 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 (LOD) of 1,000 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 post-testing to query for symptom development. No follow-up was required for SARS-CoV-2 negative participants, but all participants have secure online access to their results.

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.

Results

ACORN enrolled participants April 6, 2020 to May 16, 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 prior to arriving for testing or no-show for the swabbing appointment. SARS-CoV-2 test results were reported for 2953 participants.

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 I. Baseline characteristics, medical history, and overall infection risk factors were generally consistent between SARS-CoV-2 positive and negative participants (Table I). However, prevalence of SARS-Cov-2 infections appeared to be numerically higher for participants in the age group of 18 to <30 years of age (4.9% tested positive) (Table I). 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 I). At enrollment, participants were required to be free of new onset or worsening

fever, cough, and shortness of breath. However, additional non-specific 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 I). Proportion of positive and negative participants reporting these non-specific symptoms were generally consistent.

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 1 symptom during the 14-day follow-up period after the test date (Table II). 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 post index test date. At the time these analyses were conducted, follow-up was ongoing for the remaining participants who tested positive.

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 non-selected 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 March 24, 2020 to May 4, 2020 (May 11, 2020 for residents of Marion County [including Indianapolis]), 3.1% of asymptomatic participants tested positive for SARS-CoV-2 in ACORN (between April 7, 2020 and May 16, 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

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 pre-symptomatic. Follow-up is ongoing for remaining

participants who tested positive and differentiation between asymptomatic and pre-symptomatic 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 pre-symptomatic (28%). The symptoms reported among the pre-symptomatic participants were variable and generally mild. These prevalence and interim follow-up data contribute to our understanding of the asymptomatic and pre-symptomatic 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 pre-symptomatic individuals play in this global pandemic.

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References

1. Ghebreyesus T. General's opening remarks at the media briefing on COVID-19. <https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020>. Accessed May 18, 2020.
2. COVID-19 Dashboard by the Center for Systems Science and Engineering (CSSE) at Johns Hopkins University (JHU). <https://coronavirus.jhu.edu/map.html>. Accessed May 18, 2020.

3. Lipsitch M, Swerdlow DL, Finelli L. Defining the Epidemiology of Covid-19 - Studies Needed. *N Engl J Med*. 2020;382(13):1194-1196.
4. Bai Y, Yao L, Wei T, et al. Presumed Asymptomatic Carrier Transmission of COVID-19. *JAMA*. 2020;323(14):1406-1407.
5. Lavezzo E, Franchin E, Ciavarella C, et al. Suppression of COVID-19 outbreak in the municipality of Vo, Italy. 2020; MedRxiv Preprint: 10.1101/2020.1104.1117.20053157. Available at: <https://www.medrxiv.org/content/medrxiv/early/2020/04/18/2020.04.17.20053157.full.pdf>.
6. Mizumoto K, Kagaya K, Zarebski A, Chowell G. Estimating the asymptomatic proportion of coronavirus disease 2019 (COVID-19) cases on board the Diamond Princess cruise ship, Yokohama, Japan, 2020. *Euro Surveill*. 2020;25(10).
7. Rothe C, Schunk M, Sothmann P, et al. Transmission of 2019-nCoV Infection from an Asymptomatic Contact in Germany. *N Engl J Med*. 2020;382(10):970-971.
8. Centers for Disease Control and Prevention. Influenza Specimen Collection. 2014; <https://www.cdc.gov/flu/pdf/freeresources/healthcare/flu-specimen-collection-guide.pdf>. Accessed June 4, 2020.

9. Centers for Disease Control and Prevention. CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time PCR Diagnostic Panel. 2020; <https://www.fda.gov/media/134922/download>.
10. Back on Track Indiana - Preliminary Results - May 13, 2020. 2020; <https://fsph.iupui.edu/doc/preliminary-results-random-sample-testing-may-13.pdf>. Accessed May 18, 2020.
11. United States Census Bureau. Quick Facts - United States. 2019; <https://www.census.gov/quickfacts/fact/table/US/PST045219>.

Tables

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

	Positive N=91	Negative N=2862	Overall N=2953
Age, years, mean (SD)	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)
Pre-existing 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)

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)

^aExcluding self

^bEmphysema or Chronic Obstructive Pulmonary Disease

^cPercentage calculated relative to number of female participants

^dPercentages for infection risk factors may not add to 100% because participants were allowed to select multiple risk factors

^eWithin the past 14 days

^fAmong 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).

^gAmong 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).

^hAmong 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)

ⁱNausea, vomiting, or diarrhea

BMI, body mass index (kg/m²); CI, confidence interval; COVID-19, Coronavirus Disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SD, standard deviation

Table II. Symptoms development 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 $\geq 100^{\circ}\text{F}$)	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)

^aNausea, vomiting, or diarrhea