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BRIEF REPORT

Asymptomatic Summertime Shedding of Respiratory Viruses

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To determine rates of both symptomatic and asymptomatic infection among ambulatory adults, we collected nasopharyngeal swab specimens, demographic characteristics, and survey information from 1477 adult visitors to a New York City tourist attraction during April–July 2016. Multiplex polymerase chain reaction analysis was used to identify specimens positive for common respiratory viruses. A total of 7.2% of samples tested positive for respiratory viruses; among positive samples, 71.0% contained rhinovirus, and 21.5% contained coronavirus. Influenza virus, respiratory syncytial virus, and parainfluenza virus were also detected. Depending on symptomatologic definition, 57.7%–93.3% of positive samples were asymptomatic. These findings indicate that significant levels of asymptomatic respiratory viral shedding exist during summer among the ambulatory adult population.

Key words. Asymptomatic infection; respiratory viruses; viral shedding; definition of symptomatic infection; common cold questionnaire.

Much of the surveillance for respiratory virus infections in humans is conducted through networks of clinics and hospitals performing patient services. Specifically, patients presenting with influenza-like illness or another nonspecific illness are recorded at medical clinics and emergency departments, and these numbers are reported to county and state health agencies [1, 2]. Specimens are also obtained from some individuals presenting with influenza-like illness or similar symptoms and tested for the presence of respiratory virus, using a rapid diagnostic or laboratory assay. These data provide an estimate of respiratory virus infection rates among those seeking medical attention. However, these surveillance systems, owing to their passive form, do not capture infection rates among the population of individuals who do not seek medical attention.

These latter, unrepresented individuals—persons not seeking medical attention—constitute a generally undocumented population experiencing asymptomatic or symptomatic respiratory virus infection. Such persons may be wholly unaware of their infection, experience mild symptoms, or become iller but choose not to seek medical attention. Here, we explore respiratory virus infection rates in a segment of this population through a convenience survey and sampling study of adult visitors to a New York City tourist attraction during spring and summer 2016.

METHODS

We solicited participants from among visitors to the New York City tourist attraction during 29 April–31 July 2016. The location, a collection point for both tourists and locals, provides a cross-section of potential participants who are broadly representative of the local and visiting populations of New York City. All activities, including participant solicitation, consenting, surveying, and sampling for respiratory viruses, were performed on weekends at the attraction. Adults aged ≥18 years who were interested in participating were provided a detailed study description and consent form (Columbia University Medical Center Institutional Review Board [IRB] approval AAAQ4358; American Museum of Natural History IRB approval FWA00006768). Consenting adults were then administered a baseline survey and 2 nasopharyngeal swab samples, one from each nostril, were collected.

Survey

Participants were asked to provide information on their age, race, sex, recent travel, and preexisting medical conditions, including seasonal allergies, as well as a rating of 9 current symptoms commonly related to respiratory tract infection (see the Supplementary Materials for the full survey) per the Common Cold Questionnaire [3, 4]. These symptoms—fever, chills, muscle pain, watery eyes, runny nose, sneezing, sore throat, cough, and chest pain—were recorded on a Likert scale (ie, none, mild, moderate, or severe); each individual symptom was then quantified on the basis of these designations (ie, 0 for none, 1 for mild, 2 for moderate, and 3 for severe), and a total symptom score was tallied by summing all 9 symptoms values (range, 0–27).

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Specimen Collection and Analysis

The nasopharyngeal samples were collected using minitip flock swabs (VWR catalog no. 10755-196; Copan Diagnostics). Both samples were stored jointly in 2 mL of DNA/RNA Shield (product no. R1100-250; Zymo Research) at 4°C–25°C for up to 30 days and then were aliquoted into two 2-mL cryovials and stored at –80°C. Nucleic acids were extracted from 200 µL of thawed sample, using the EasyMAG NucliSENS automated system (bioMerieux). Reverse transcription–polymerase chain reaction analysis amplification was performed using the Veriti 96-well thermal cycler (Applied Biosystems) per the GenMark package instructions. Samples were then subjected to GenMark's RVP Exonuclease polymerase chain reaction program, transferred to a GenMark RVP cartridge, and loaded into the eSensor for measurement of signal intensity, per manufacturer protocols.

The GenMark eSensor RVP system separately detects influenza A virus of any subtype, influenza A(H1N1), influenza A(H3N2), 2009 pandemic influenza A(H1N1), influenza B virus, respiratory syncytial virus A and B, parainfluenza virus 1–4, human metapneumovirus, human rhinovirus (HRV), adenovirus B/E and C, coronavirus (CoV) 229E, CoV NL63, CoV OC43, and CoV HKU1. The eSensor system measures electrical signal intensity in nanoamps per millimeter squared. Per manufacturer specifications, samples positive for a particular virus were identified by an intensity of ≥3 nA/mm^2.

Definitions of Asymptomatic Infection

To test the sensitivity of our findings, because no standard definition of symptomatic infection exists, we used multiple definitions to delineate symptomatic from asymptomatic participants. The first symptomatic classification (definition 1) required self-report of ≥2 symptoms, with at least 1 being moderate or severe [5]. Definition 2 relaxed this standard and required that only ≥1 symptom was moderate or severe. Only fever, cough, and sore throat, the symptoms used to diagnose influenza-like illness [1], were used to delineate the remaining 3 symptomatic definitions. For definition 3, a symptomatic participant had to self-report fever, cough, or sore throat as moderate or severe. A mild or worse fever and a mild or worse cough or sore throat was needed to be symptomatic according to definition 4. Definition 5 required a mild or worse fever and a moderate or worse cough or sore throat.

Statistical Analysis

While the research protocol was exploratory, we hypothesized that shedding participants would more likely be symptomatic and that travel and age would be associated with shedding. Statistical differences in symptom scores among categorical groupings (eg, age and race) were assessed using analysis of variance (ANOVA) and the Tukey test, whereas differences across pairs of categorical variables (eg, being symptomatic and positive for infection) were assessed using χ^2 analysis and the Fisher exact test. Associations between signal intensity among positive samples (all positive samples, HRV-positive samples, and CoV-positive samples) and race, sex, age category (18–29, 30–39, 40–49, 50–64, and ≥ 65 years), allergies, travel, residence, and Hispanic self-identification were described using univariate and multivariate regression. Best-fit models were identified using the Akaike information criterion. Similarly, logistic regression was used to identify factors associated with a virus-positive sample (ie, positive vs negative).

RESULTS

Demographic Characteristics

We consented, surveyed, and swabbed 1477 adults between 29 April and 31 July 2016. A total of 57.4% of participants were female, 41.8% were male, and 0.8% responded either "transgender," "gender nonconforming," or "don't know." There were 67.7% of participants who self-identified as white, 14.8% as Asian, 3.6% as black/African American, 1.5% as American Indian/Alaska Native, 0.3% as Native Hawaiian/Pacific Islander, and 5.8% as another race, and 6.1% gave no response. A total of 22.3% self-identified as Hispanic. The age distribution was as follows: 38.5% were aged 18–29 years, 20.9% were aged 30–39 years, 20.4% were aged 40–49 years, 13.9% were aged 50–64 years, and 4.5% were aged ≥65 years. More than half (53.6%) of participants received an influenza vaccine in the preceding year.

Viral Results

A total of 7.2% of samples (107 of 1477) tested positive for respiratory virus. Of the positive samples, 71.0% (76) were HRV positive, 21.5% (23) were CoV positive, and 7.4% (8) were positive for human metapneumovirus, influenza virus, respiratory syncytial virus A, and parainfluenza virus 2–4 (Table 1). Virus-positive participants were identified throughout the study period; however, rates of positivity were lower in July than during April, May, and June. No coinfections were detected.

Table 1. Percentage of Samples Positive for Respiratory Virus, Overall and by Virus

Virus	Positive, %
All samples (n = 1477)	7.2
Virus-positive samples (n = 107)	
Coronavirus	21.5
Human metapneumovirus	1.9
Human rhinovirus	71.0
Influenza virus	0.9
Human respiratory syncytial virus	0.9
Parainfluenza virus 2	0.9
Parainfluenza virus 3	1.9
Parainfluenza virus 4	0.9

Symptom Results

Across all participants, women reported significantly higher total symptom scores than men ($P < .024$ by ANOVA), and participants aged 30–39 years, 40–49 years, and 50–64 years reported significantly lower total symptom scores than participants aged 18–29 years ($P < .05$ by the Tukey test). In addition, consumption of cold and influenza medicines was significantly positively associated with higher total symptom scores ($P < .0001$ by ANOVA), and this association held when the analysis was restricted to those testing positive for respiratory virus ($P = .0001$ by ANOVA).

Among all participants there was a statistically significant positive association between reporting a greater tendency to get sick and total self-reported symptom score ($P < .0001$ by the Tukey test); however, there was no significant association between reporting a greater tendency to get sick and actual detection of respiratory virus shedding ($P = .10$ by χ^2 analysis).

Virus-Positivity Analysis

Testing positive for respiratory virus (≥3-nA/mm^2 signal intensity) was positively associated with consumption of cold and influenza medicine ($P < .0001$ by the Fisher exact test). There was no association between testing positive and having received influenza vaccine during the previous year, recent travel, or location of residence. Among participants testing positive, 6.7%–42.3% qualified as symptomatic, depending on the definition used (Table 2). Rates of being symptomatic differed significantly among virus-positive and virus-negative participants ($P < .002$ for all comparisons by χ^2 analysis and the Fisher exact test) with positive participants more likely to qualify as symptomatic.

The best-fit logistic regression model supported an association between an increased likelihood of testing positive for respiratory virus infection and a higher total symptom score ($P < .0001$) and being Hispanic ($P < .005$). A similar association was found for the likelihood of testing positive for HRV. Only a higher total symptom score ($P = .001$) was associated with an increased likelihood of testing positive for CoV.

Quantitated Score Analysis

Among those testing positive for respiratory virus, the best-fit model revealed a statistically significant negative association between GenMark eSensor RVP signal intensity and the age categories 40–49 years and 50–64 years ($P = .036$ and $P = .003$, respectively) relative to the age category 18–29 years, and a positive association with seasonal allergies ($P = .034$). When only the HRV signal intensity was regressed, a statistically significant negative association with the age category 50–64 years ($P = .038$) relative to the age category 18–29 years emerged. Regression of the CoV signal intensity revealed a more complex positive association with total symptom score ($P = .007$) and negative associations with self-identification as black and American Indian/Alaska Native ($P = .003$ for both), relative to white, and with the age category 50–64 years ($P = .002$), relative to the age category 18–29 years.

DISCUSSION

Here, we found that 7.2% of adult participants visiting a New York City tourist attraction during late spring and summer tested positive for a common respiratory virus. Depending on the definition used, 57.7%–93.3% of those testing positive qualified as asymptomatic. The asymptomatic percentages derived using definitions 1 and 2 (59.6% and 57.7%, respectively), which had laxer criteria, fell within the broad range (9%–80%) found in prior studies [6–9]. Definitions 3–5, which used more-stringent influenza-like illness symptom criteria, yielded higher asymptomatic percentages. Twenty-six percent of participants testing positive reported a total symptom score of 0. In contrast, 83.0%–98.7% of participants testing negative for a common respiratory virus qualified as asymptomatic, depending on the definition used.

Regardless of symptom definition, all participants testing positive were ambulatory and taking the time to visit the tourist attraction. However, the differences suggest that people experiencing influenza-like illness symptoms, in particular fever, may be more apt to stay home. Indeed, fever was the least commonly reported of the 9 surveyed symptoms.

Table 2. Percentage of Symptomatic and Asymptomatic Infections Among Individuals With Samples Positive or Negative for Respiratory Viruses, by Definition of Asymptomatic Infection

		Positive, %		Negative, %			*P*		
Definition	Symptom Score Summary, Median (IQR)	Symptomatic	Asymptomatic	Symptomatic	Asymptomatic	χ^2 Statistic	By the χ^2 Test	By the Fisher Exact Test	OR (95% CI)
1	5 (4–8)	40.4	59.6	13.3	86.7	54.861	<.0001		4.42 (2.88–6.74)
2	5 (3–7)	42.3	57.7	17.0	83.0	40.188	<.0001		3.57 (2.35–5.39)
3	7 (4–9)	20.3	79.8	5.7	94.3	31.893	<.0001		4.17 (2.40–7.01)
4	10 (7–13)	7.7	92.3	1.6	98.4	18.112	<.0001	.0007	5.17 (1.93–12.54)

See Methods for definitions of asymptomatic infection.

Among those testing positive, for either all viruses or HRV alone, we found an association between eSensor signal intensity and participant age. In addition, signal intensity was positively associated with self-reported total symptom score for CoV. Previous studies, as well as manufacturer specifications, have indicated little association between eSensor signal intensity and the amount of virus present [10, 11]. The finding here that symptom severity and shedding of CoV are positively associated warrants further investigation.

The current findings, based on a large, diverse sample of ambulatory adults, provide a baseline prevalence of respiratory virus shedding among this subpopulation during the northern hemisphere summer in a major city, where tourists abound. It is unclear how this shedding among approximately 1 in 14 adults contributes to the transmission of these pathogens. Indeed, while the nasopharyngeal specimens document shedding, the contagiousness of the participants is unclear. Rhinovirus and coronavirus were most prevalent. While their greater abundance might indicate that these 2 viruses are more communicable, it is possible that their higher prevalence is linked with immune escape or innate transmissibility.

Our sampling scheme introduces some biases that may make the findings not reflective of shedding across the entire population; specifically, very ill individuals stay home, and those feeling symptoms might have been more willing to participate in this study. Participants self-reported fever, whereas a thermometer measure might have provided more-definitive data. Further, participants only reported symptoms over the last 48 hours; however, for some respiratory viruses, RNA can be detected weeks following acute illness. Thus, we cannot fully determine whether asymptomatic positive samples were due to prior illness, represented an incubating infection, or were truly asymptomatic.

The findings clearly indicate that substantive levels of asymptomatic shedding exist among the sampled adult ambulatory population. It will be important to repeat this study during the winter cold and influenza season and determine how overall infection rates, infection rates by virus, and asymptomatic infection rates vary from summer to winter. The findings presented here can be used to complement findings from household surveys, to improve estimates of virus infection incidence, and to inform model simulation, forecast, and control of infections due to these pathogens. In particular, medical countermeasures might be deployed more effectively if the true scope of respiratory virus infection incidence in the population were better understood.

Notes

Disclaimer. The views, opinions and/or findings expressed are those of the authors and should not be interpreted as representing the official views or policies of the Department of Defense or the US government.

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