


# Epidemiological study of people living in rural North Carolina for novel respiratory viruses

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## Funding information

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## Summary

During the last 10 years, scientists have grown increasingly aware that emerging respiratory viruses are often zoonotic in their origin. These infections can originate from or be amplified in livestock. Less commonly recognized are instances when humans have transmitted their respiratory pathogens to animals (reverse zoonoses). Even with this knowledge of viral exchange at the human–livestock interface, few studies have been conducted to understand this cross-over. In this pilot study, we examined persons with influenza-like illness at an outpatient clinic for evidence of infection with novel zoonotic respiratory pathogens in rural North Carolina where there are dense swine and poultry farming. Environmental air sampling was also conducted. From July 2016 to March 2017, a total of 14 human subjects were enrolled and sampled, and 192 bioaerosol samples were collected. Of the 14 human subject samples molecularly tested, three (21.4%) were positive for influenza A, one (7.1%) for influenza B and one (7.1%) for human enterovirus. Of the 192 bioaerosol samples collected and tested by real-time RT-PCR or PCR, three (1.6%) were positive for influenza A and two (1.0%) for adenovirus. No evidence was found for novel zoonotic respiratory viruses.

## KEYWORDS

influenza-like illness, North Carolina, respiratory viruses, surveillance, zoonoses

## 1 | INTRODUCTION

Over the last decade, there has been a rapid increase in the incidence of emerging infectious diseases (EIDs) among humans, posing a serious threat to public health (Jones et al., 2008). Many of these EIDs, particularly respiratory viruses, have been found to be zoonotic in their origin (Morens, Folkers, & Fauci, 2004) and are often only identified in humans after a spillover from the animal reservoir has occurred. Such examples include avian influenza viruses, swine influenza viruses, severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV) and Ebola virus (Wang & Cramer, 2014). Given that zoonotic viruses have repeatedly caused serious disease outbreaks throughout the world, they are considered to be the most likely aetiological agents for the next pandemic (Morse et al., 2012). Hence, understanding the

epidemiology of viral zoonotic diseases, especially in environments where humans and animals have close contact, is important for prevention efforts.

In the United States, agricultural livestock production has been associated with the occurrence of zoonotic virus transmission. More recently, there has been an outbreak of swine influenza variant (H3N2v) virus transmitted from pigs to humans at agricultural fairs in the Midwest, resulting in a total of 376 human cases as of 2 August 2017 (Centres for Disease, 2017). There has also been evidence to suggest an earlier peak in influenza virus transmission among populations residing in geographical areas with a higher density of swine farms (Lantos, Hoffman, Hohle, Anderson, & Gray, 2016). However, even with this knowledge of potential viral exchange, the human–livestock interface is seldom studied, and often only when an outbreak has already occurred. One barrier to implementing routine surveillance at

the human–livestock interface is the concern that identifying a novel agent might have a negative economic impact on production.

In this pilot study, we attempted an alternative surveillance strategy for the detection of novel respiratory viruses, where we enrolled humans with influenza-like illness seeking treatment at an outpatient clinic located in rural North Carolina where pig and poultry production were dense. Environmental air sampling in the clinic was also performed.

## 2 | MATERIALS AND METHODS

### 2.1 | Ethics approval and study location

This study was approved by the institutional review board at Duke University. Permission was obtained from CommWell Health to conduct this study at one of their outpatient clinics located in rural North Carolina where there is a high density of swine and poultry farming.

### 2.2 | Subject recruitment and enrolment

Study personnel from Duke University travelled to the clinic one-two times per week to enrol participants. Individuals older than 2 years of age who sought medical care at the clinic were recruited for participation in the study if they met the following inclusion criteria: (i) an influenza-like illness (acute onset of a respiratory illness with a temperature, measured at the clinic or self-reported in 72 hr prior to visit, greater than or equal to 38°C, accompanied by a cough or sore throat for 4 or more hours) or (ii) radiographic evidence of pneumonia. Individuals who met the inclusion criteria were consented (parental consent required for adolescents 2–17 years old and assent for adolescents 12–17 years old) and investigated to complete a brief questionnaire about their health, living and working environments, as well as their exposure to poultry (chickens, ducks, geese, turkeys and other) or swine, which was defined as close contact (touching or within 1 m) in the past 30 days or 12 months.

### 2.3 | Subject sample collection

Each participant permitted the collection of two nasal swab specimens (one per each nostril), which were placed in viral transport media (BD Diagnostics, Franklin Lakes, NJ), placed on wet ice, and transported the same day to the Duke One Health Research Laboratory. Samples were stored at –80°C until molecular testing was performed.

### 2.4 | Bioaerosol sampling

Bioaerosol sampling was conducted by study personnel during each clinic visit in four of the triage rooms where patients were medically screened before being moved to an exam room. Air samples were collected using filter cassettes with polytetrafluoroethylene (PTFE) filters (0.3- $\mu$ m pore, 37 mm) connected to an AirCheck XR5000 personal sampling pump (Cat #: 210-5000; SKC, Inc., Eighty Four, PA, USA). The filter cassettes were placed in the same location in each

#### Impacts

- Novel zoonotic viruses can emerge when animals and humans have close contact.
- Current surveillance approaches are not designed to efficiently and reliably identify novel viruses of zoonotic origin before an outbreak occurs.
- New surveillance approaches that are conducted in partnership between the human health, animal health and animal production sectors, and which incorporate modern sampling techniques to mitigate disruptions to production, are needed.

room, approximately 1.5 m above the ground, between where the medical provider and patient would sit. The sampling pumps were calibrated to a flow rate of 5 L/min and allowed to run for approximately 120–180 min. At the end of the sampling period, study personnel recorded the room number, minutes of sampled air, date, time and sampler number. Cassettes were then placed on wet ice and immediately transported back to the Duke One Health Research Laboratory where the filters were removed, swabbed with a flocked swab pre-wetted with sterile virus collection medium (PBS with 0.5% w/v BSA fraction V), and eluted into 0.5 ml of the same virus collection medium. Samples were stored at –80°C until molecular testing was performed.

### 2.5 | Laboratory testing

Viral DNA was extracted from nasal swab and bioaerosol samples using a QIAamp DNA Blood Mini Kit (QIAGEN, Inc., Valencia, CA) and tested with a real-time PCR (qPCR) assay for adenovirus (Bil-Lula, De Franceschi, Pawlik, & Wozniak, 2012) using a QuantiNova Probe PCR kit (QIAGEN, Inc.). Ad-positive specimens were subtyped using gel-based screening PCR targeting the hexon gene (Lu & Erdman, 2006). Viral DNA was also assessed using gel-based PCR assays with the Platinum<sup>®</sup> Taq DNA Polymerase Kit (Thermo Fisher Scientific, Inc., Waltham, MA) for the detection of pan-species adenovirus (Wellehan et al., 2004).

Viral RNA was extracted from nasal swab and bioaerosol samples using the QIAamp Viral RNA Mini Kit (QIAGEN, Inc.) and then assessed with real-time RT-PCR (qRT-PCR) assays using the SuperScript<sup>®</sup> III Platinum One-Step qRT-PCR System with Platinum<sup>®</sup> Taq DNA Polymerase (Thermo Fisher Scientific, Inc.) for the detection of influenza A (WHO, 2014), influenza B (Selvaraju & Selvarangan, 2010), influenza C (Pabbaraju et al., 2013), influenza D (Hause et al., 2013), human coronavirus (Loens et al., 2012) and human enterovirus (Oberste et al., 2013). Viral RNA was also assessed with gel-based RT-PCR assays using the SuperScript<sup>®</sup> III Platinum One-Step RT-PCR System with Platinum<sup>®</sup> Taq DNA Polymerase (Thermo Fisher Scientific, Inc.) for the detection of pan-species enterovirus (WHO, 2015) and pan-species coronavirus (unpublished).

**TABLE 1** Characteristics of enrolled participants

Characteristics	Total N (%)
Total	14 (100)
Sex	
Female	9 (64.3)
Male	5 (35.7)
Age groups	
<20	7 (50.0)
20–30	3 (21.4)
30–40	2 (14.3)
>40	2 (14.3)
Household size	
1–2	3 (21.4)
3–5	8 (57.2)
>5	3 (21.4)
Children under 5 years of age at home	
Yes	10 (71.4)
No	4 (28.6)
Primary language	
Spanish	10 (71.4)
English	4 (28.6)
Occupation <sup>a</sup>	
No occupation	8 (57.1)
Other	6 (42.9)
Animal exposure in the last 30 days <sup>b</sup>	
Pigs	2 (14.3)
Chickens	3 (21.4)
No exposure	9 (64.3)
Animal exposure in the last 12 months <sup>c</sup>	
Pigs	2 (14.3)
Chickens	3 (21.4)
No exposure	9 (64.3)
History of antibiotic taken in the last 90 days	
Yes	3 (21.4)
No	11 (78.6)
History of hospitalization in the last 90 days	
Yes	1 (7.1)
No	13 (92.9)

<sup>a</sup>Other category includes agricultural, healthcare services, education, law and social, community and government services, metal manufacturing, sales and service occupations.

<sup>b</sup>Defined as touching or within 1 m; two individuals reported exposure to both poultry and pigs.

<sup>c</sup>Defined as touching or within 1 m; two individuals reported exposure to both poultry and pigs.

### 3 | RESULTS

From July 2016 to March 2017, a total of 14 human subjects meeting the case definition were enrolled in the pilot study

(Table 1). Most participants were female (64.3%), less than the age of 20 years old (50.0%), and spoke Spanish (71.4%). There were two (14.3%) participants who reported having close contact (touching or within 1 m) with pigs and three (21.4%) participants who reported having close contact (touching or within 1 m) with chickens in the last 30 days and 12 months. No participant had exposure to ducks, geese or turkeys. There were three participants (21.4%) who reported the use of antibiotic drugs in the last 90 days.

Nasal swab specimens were collected from all 14 participants, as well as a total of 192 bioaerosol samples from the clinic. Of the 14 nasal swab specimens collected and molecularly tested, three (21.4%) were positive for influenza A, one (7.1%) for influenza B and one (7.1%) for human enterovirus. Of the 192 bioaerosol samples collected and tested by real-time RT-PCR or PCR, three (1.6%) were positive for influenza A and two (1.0%) for adenovirus.

### 4 | DISCUSSION

In this pilot study, we conducted surveillance for human and zoonotic respiratory viruses among humans with influenza-like illness who visited an outpatient clinic located in a rural area of North Carolina where there is dense swine and poultry production. We also sampled the air in the clinic using bioaerosol sampling equipment for the same viruses. Of the fourteen individuals with ILI whom we enrolled and sampled, influenza A virus, influenza B virus and enterovirus were detected in specimens collected between 12 January 2017 and 7 March 2017. Influenza A virus was detected in the environmental air samples collected between 12 December 2017 and 31 January 2017. Additionally, we detected adenovirus in two environmental air samples collected on 23 July 2016 and 7 September 2016.

Through our surveillance, we were able to identify the presence of influenza A virus a month earlier using an air sampling approach, compared to that of samples collected from human ILI participants. It is likely that influenza A virus was circulating among the human population as early as December 2016, when it was first detected in air samples, but we were not able to identify it in nasal swab specimens as either individuals with ILI were not willing to participate in our study, or individuals did not meet the ILI case definition, but had a subclinical infection and were still shedding virus. While we did attempt to run conventional RT-PCR assays to isolate influenza A gene segments for sequencing on samples that were positive by the real-time assay, the RT-PCR assays were not successful likely because the CT values of the real-time positive samples were all >37, suggesting low concentrations of viral RNA.

Adenoviruses are DNA viruses known to be environmentally ubiquitous given their relative stability compared to RNA viruses. While we detected adenovirus in the air, we did not identify adenovirus in any of our human ILI specimens, likely for similar reasons posited for the influenza A detections. Notably, we have detected human and porcine adenovirus in similar air sampling studies

conducted in Singapore (Nguyen et al., 2017; Poh et al., 2017). While adenovirus is typically considered to have less of an impact on human health compared to other respiratory viruses such as influenza A, large community outbreaks of novel adenovirus strains causing severe respiratory infection have been well documented (Scott et al., 2016).

We did not find evidence of novel or zoonotic influenza virus, enterovirus, coronavirus or adenovirus using our pan-species molecular detection methods. It was hypothesized that individuals living in geographic areas with high poultry and pig production might serve as a sentinel population for the transmission of zoonotic pathogens. Given our limited sample size of ILI participants, we likely lacked the sample numbers necessary to detect a zoonotic transmission event, presuming such an event is rare. Additionally, some of the individuals whom we enrolled and sampled did not report any exposures to swine or poultry. Thus, it is not clear whether our surveillance approach for zoonotic viruses was effective.

Despite the limitations, results continue to reinforce the idea that air sampling could be a useful surrogate or compliment for infectious diseases surveillance in settings where it may be difficult to directly study the human population. This pilot study also underscores the need of developing close partnerships between public health, veterinary health and the animal production industry, to move towards a surveillance approach that is inclusive of animal production workers and the environments in which they work, while also mitigating the economic impact concerns production owners may have. This seems to be the optimal approach to surveillance to best capture the most reliable epidemiological data possible.

## ACKNOWLEDGEMENTS

This study was supported by Duke University discretionary funding (Gray PI). We thank Dr. Thomas Arcury, Dr. Sara A. Quandt, Dr. John W. Sanders and Dr. Werner Bischoff of Wake Forest School of Medicine, as well as Christopher Vann, Christopher Frank and the clinical nursing staff of CommWell Health for their early advice and support of this study. We also thank Jennifer Callejas, Thara Veeramachaneni and Maria Perez for their translation services and Tai Xie, Jane Fieldhouse, Sarah Philo, Laura Borkenhagen and Lifang Wang in assisting with enrolment and sample collection.

## CONFLICT OF INTEREST

None.

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**How to cite this article:** Wang X, Anderson BD, Pulscher LA, Bailey ES, Yondon M, Gray GC. Epidemiological study of people living in rural North Carolina for novel respiratory viruses. *Zoonoses Public Health*. 2018;65:e265–e269. <https://doi.org/10.1111/zph.12436>