ABSTRACT

Title of Thesis: TRACKING ACUTE RESPIRATORY
                                          INFECTIONS IN A COLLEGE RESIDENT
                                          COMMUNITY

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Influenza and other acute respiratory infections (ARIs) contribute significantly

to human morbidity and mortality globally. Animal experiments and human challenge

studies have not provided an adequate explanation about the relative importance of

social, behavioral and physical environment in the transmission of ARIs and are

limited due to uncertainty about the generalizability of their findings to a natural

infection. Also, household transmission studies seldom characterize all potential

transmission covariates e.g. environmental conditions, leaving a gap in the knowledge

of transmission mechanisms. Here, we describe the design and preliminary results of

an extensive college dormitory ARI transmission study that has the potential to

characterize several important ARI transmission covariates; we critically appraise the

design and show how the findings from such design can be applied to answer most of

the vital questions that exist about the transmission of ARIs.

TRACKING ACUTE RESPIRATORY INFECTIONS IN A COLLEGE

RESIDENT COMMUNITY

by

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Chapter 1: Introduction

Problem significance

Despite huge efforts being expended towards the prevention and control of acute

respiratory infections (ARIs), they still pose a significant burden on healthcare and

the economy. ARIs such as common cold and pharyngitis, represent one of the

biggest single contributors to the overall burden of disease in the world as measured

by disability-adjusted-life-years (DALY) lost (1–3). The DALY for a disease or

health condition is the sum of the years of life lost from premature mortality in the

population and the years lost from disability in people living with the health condition

or its consequences (4). Globally, about 4.5 million people die from ARIs yearly, and

ARIs account for about 100 million hospitalizations leading to expenditure of

millions of dollars (1,5). During yearly influenza epidemics in the United States,

about 50,000 influenza-related deaths have occurred from 1976 to date. (6)

Broadly, ARIs are caused by bacterial or viral pathogens. Bacterial pathogens are

less-common causes of ARIs but can include *Streptococcus pneumonia, Mycoplasma*

pneumonia, Haemophilus influenza, *Chlamydophila pneumonia, Coxiella*

burnetii and *Legionella pneumophila especially in immunocompromised individuals*

(7). Viral causative agents of ARIs include rhinoviruses, respiratory syncytial virus,

influenza virus, parainfluenza virus, human metapneumovirus, adenovirus, and

coronaviruses (8). Though most viral ARIs are self-limiting, very large studies (9,10)

have shown that they even contribute more than other infectious agents to the

morbidity and mortality due to ARIs. Viral ARIs can occur in epidemics and can

spread very rapidly within communities across the globe. For example, the World Health Organization (WHO) reports that every year, influenza virus causes respiratory tract infections in 5–15% of the population and severe illness in 3–5 million people leading to between 290,000 and 650,000 deaths (11,12). In 2003, severe acute respiratory syndrome (SARS), a novel strain of coronavirus spread very rapidly throughout the world leading to thousands of deaths (13). Also, respiratory infections due to viral pathogens may predispose to more serious respiratory complications such as bacterial sinusitis, bronchitis, or pneumonia. The 2017 – 2018 influenza season was characterized predominantly by the circulation of the influenza A (H3N2) virus which has been observed to cause more severe illnesses than H1N1 and Influenza B viruses (14). Expectedly, hospitalization rates in the last season were high and so was the number of mortalities recorded especially for children and the elderly. Though similarities abound between the clinical syndromes of various respiratory viruses, they may possess differing transmission patterns. Understanding the predominant mode or modes of transmission of each of these infectious agents from person-to-person is crucial to the understanding of how they behave in environments and the impact that various social, physical and behavioral environmental factors have on their transmissibility. All these put together will greatly enhance the development of a conceptual basis for designing optimal control strategies.

Chapter 2: Literature Review

Relevant theory

In the scientific world, there is no consensus about the modes of transmission of these individual viral agents. Pica et al (15) provided a classification for modes of transmission of ARIs which defines contact transmission as direct and indirect while airborne comprises both droplet spray and aerosol modes. Contact transmission (both direct or indirect) arises from contact with pathogen-containing droplets: direct contact transmission refers to physical contact and transfer of pathogens from an infected person to a susceptible person, but indirect contact transmission refers to contact with fomites and subsequent transport of the pathogen via, for example, hands to the upper respiratory tract (16). When large droplets are generated by coughing and sneezing, they can get deposited immediately onto the mucous membrane of susceptible people, this is known as droplet transmission (16). These large droplets gravitationally settle quickly hence droplet transmission constitutes a transmission mode that is mainly significant for close contacts (16). Airborne transmission occurs via inhalation of very small respiratory droplets (aerosols) that are small enough to remain airborne (17).

Since the first major influenza outbreak - the "Spanish Flu" of 1918 which led to the death of about 50 million people worldwide, scientists have been investigating the modes of transmission of ARIs. While great discoveries have been made, there are still numerous knowledge gaps regarding the transmission and possible control

strategies and ever since then, different methods of study have been designed to try to answer the lingering questions.

Experimental infection of animals or healthy volunteers has been an important method of studying ARIs for several reasons including that they provide unique opportunities to describe the progressive course of the illness from the onset as well as the shedding and symptoms characteristics prospectively. They also offer a controlled environment making it easier to study the impacts of environmental factors on transmission. Perhaps because of these, many early studies on influenza were experimentally done on animals and humans with the aim of observing the effects of various conditions on the transmission (18,19) . In an early human experimental study following the 1918 pandemic, upper respiratory secretions from infected humans that were collected during the pandemic in the active phase of their infections were inoculated into the nostrils of a group of sailors who were onboard during the outbreak and who were thought to have had no prior exposure to the virus. In the same study, blood samples from infected influenza patients were subcutaneously injected onto another group of sailors. In both experiments, no evidence of disease was observed in the subjects. The results from these experiments brought to the front, important questions about the transmission of the deadly virus, questions such as, "What is the point of entry of the virus? "What is the mode of transmission and when is the most infectious time in the clinical course of the disease?" Subsequent studies, including experimental studies, helped to shed more light on some of these queries. Careful intranasal liquid inoculation of the viral particles and in some cases, aerosol inoculation have successfully produced infections in mice, guinea pig, ferrets, and

non-human primates. (20–24) However, there remains the concern that the spectrum

of disease observed with artificial infections and transmission pattern and infections'

loci may differ from that in natural infections thus limiting the external validity of

these studies. Nevertheless, these studies have gone to show the importance of

different modes of infection in transmission, but up to date, the relative importance of

each of them is still unclear for different pathogens. The reasons for these varieties

could include the differences in the virion structures of the viruses influencing the

pervasiveness of the particles, the immunogenicity of the viruses, the clinical

syndromes such as the quantity of nasal secretions in the infected people

(15,25). RSV and Adenovirus, for example, have been suggested to be primarily

spread by contact (direct and indirect) transmissions (15). In one study by Hall et al

(26), where 3 categories of subjects were made to come in contact with a highly

symptomatic infant with heavy secretions, one category of subjects played with the

infant, changed the child's diapers, another category touched the infant' environment

but not the infant while a third category sat next to the infant for 3 hours without

making any contact. Five of the 7 who cuddled the infant, 4 of 10 who touched and

none of 14 who sat developed RSV infection. Possible explanations for the striking

findings are that infants with RSV excrete a significant number of viral particles in

their nasal secretions for days and RSV is now known to survive well on

impermeable surfaces, skin, and gloves, for many hours thus providing opportunities

to contaminate hands. In another study, researchers detected viable infectious

particles of RSV on impactors placed 1m and 5m from some infected children's cots

in a hospital where they were being managed for RSV infection, thus suggesting that

RSV could be transmitted via aerosols (27). Similarly, varied transmission patterns and modes have been observed for Adenovirus and Coronavirus respiratory infections (28–30).

To further investigate transmission dynamics, studies have been conducted in households or families and communities because a significant number of ARI transmission occur within these environments during epidemics (31) with the rationale that the improved knowledge of the transmission dynamics of each ARI within families and in the community will accelerate the development of effective control strategies to reduce its burden. Hence, households studies have increasingly gained attention. (32). In these studies, households are recruited prospectively from a sampling frame that includes the whole community, then participants are followed up prospectively to identify infections and illnesses. Household contacts of ill participants are then followed up as well to identify secondary illnesses. Several follow-up studies of families during one or several consecutive influenza seasons have described the occurrence and spread of infections in households in relation to age, family composition, crowding, circulating viral strains, exposure in the community, and prior immunity. During the 2009 influenza pandemic, household studies were used to provide early estimates of transmission dynamics of the novel viral strain H1N1 in confined settings (33–35). Household contacts are easy to identify and follow up and they provide a well-defined number of susceptible or exposed people. They have also provided a means to evaluate intervention strategies such as anti-viral medications and vaccination (36–38) and facemasks and hand hygiene (39,39). Through household studies, more information about transmission

dynamics has been provided including "serial intervals", "secondary attack rates" or "secondary infection risks" (32,34–37,40–43), all of which are important when designing mechanistic models for infectious diseases and are important when predicting characteristics of epidemics. The serial interval is the time from the onset of illness in the index case to the time of onset of illness in the secondary cases while the secondary infection risk is the number of secondary cases divided by the number of exposed contacts (31). Household studies are limited, however, one for the huge amount of resources and logistics required to conduct them, also because it is difficult to estimate the total number of secondary infections due to one index case since some transmissions occur outside the household settings. In addition, in typical household studies, index cases are recruited after presenting with illnesses and as such recruitment may be selective for serious-illness-causing infections. If less serious illnesses are associated with greater transmissibility, then household studies are likely to underestimate transmissibility.

Other studies have been conducted in "total institutions". A total institution is a place of work or residence where many people who are similarly situated are cut off from the larger community for a significant time and together lead an enclosed life and make frequent contacts (44). Examples of such include military barracks, ships, hospitals, nursing homes, and college dormitories. Outbreaks have been reported to be particularly high in such institutions and epidemiological investigations are often carried out there (45–47). Also, outbreaks have been particularly worrisome in schools and dormitory environments where close and crowded living conditions and environmental challenges (48) are main issues. Total institutions offer great benefit

because they provide unique opportunities for infections to be transmitted and studied thus offering a classic example of how social networks can affect the transmission of infectious disease since the role of social network in transmissibility has been identified and emphasized (49–53). Also, total institutions have been valuable in the evaluation of intervention strategies such as vaccination, isolation, quarantine, and assessment of their potential usefulness in curbing transmission (50,54).

Study aims

A novel study conducted in residence halls at the University of Maryland in the Spring of 2017 is described here. The study offers a wealth of data to examine biological and social markers of susceptibility to infection and contagiousness among community-acquired cases and their closest contacts on campus. The dormitory environment provides a unique and powerful setting in which to investigate transmission and contagiousness because it represents a microcosm of greater social communities while facilitating observation of tight-knit social networks. Focus on a living-learning community with a concentration on health not only means that the population under study includes numerous close social network connections because of shared courses, dorm space, and social activity, but also the possibility that health-minded students may be more interested in the research and sets the groundwork for a more community-based participatory model that might facilitate high participation levels and quick reporting of illnesses and close contacts. Furthermore, the dormitory environment under study provides findings interpretable to the military barrack and hospital settings. Our objective here is to describe the methods employed in this

study. We also describe the cohort, their infections, and some preliminary data about

their connectivity. Finally, we will critically appraise the college dorm study and

suggest ways by which it may be improved upon.

Chapter 3: Methods

We performed a prospective study to identify and examine the transmission of influenza and other acute respiratory infections within a selected cohort of college dormitory students.

Overall study group eligibility

The target study group comprised students of University of Maryland at least 18 years of age who were in their first year and lived on certain floors in one of the dormitories assigned to a university living-learning program. The university living-learning program is a selective program that brings together scholarly students with a common academic interest to a specialized residential community, providing for them curricular and co-curricular activities that are specially designed for them. Students in this community take a series of courses together during their first two years in the university, participate in field trips and social events as members of the community, and the majority live together in a single dormitory.

For ease of surveillance, we limited participation-eligibility, to students in one of the living-learning programs. Most of the students in that program lived on 3 floors on one wing of the dormitory, but to promote equity and capture relevant social networks, those not in the living-learning program but who lived on any of those three floors were eligible to participate in the study. The overall cohort was thus divided into main and peripheral cohorts. Total number of eligible and enrolled participants within individual divisions are shown in Table 1.

Table 1. Target group showing the divisions of the students in the overall cohort.

	Location of Residence					
	Target Floors		Other		Total	
	Eligible (N)	Enrolled N (%)	Eligible (N)	Enrolled N (%)	Eligible N	Enrolled N (%)
LLC Student with LLC roommate	Main Cohort (63)	43 (68%)	Main Cohort (15)	6 (40%)	97	48 (49.5%)
LLC student without LLC roommate			Peripheral Cohort (19)	5 (14.7%)		
Non-LLC roommate of an LLC student	Main Cohort (26)	7 (26.9%)	Peripheral Cohort (1)	-	27	13 (48%)
Other students on targeted floor	Main Cohort (22)	7 (31.8%)	-	-	22	7 (31.8%)
Total	111	57 (51.4%)	35	11 (31.4%)	146	68 (46.6%)

Abbreviation: LLC = Living Learning Community
This table describes the target group of students and their divisions within the overall cohort. Main cohort indicated by orange colored cells and the peripheral cohort indicated by grey colored cells. Students in the main cohort could be evaluated as self-reported cases or contacts while those in the peripheral cohort could only be evaluated as contacts.

The size of the overall eligible study cohort was 146. There were 97 students in the scholars' program and 49 others who were either their roommates or resided on the same floors as them.

Study branches, specific branch eligibility, and recruitment

Students in the main cohort were eligible to be evaluated as either self-reported cases or contacts but those in the peripheral cohort were only eligible to be evaluated as contacts.

We obtained a list of all eligible participants, their room numbers, and their institutional e-mail addresses from the University Registrar. We sent emails leading up to the beginning of the study to inform them about the study purposes and procedures. A unique URL address was emailed to each eligible participant to invite them to enroll in the study and to get them to provide baseline information and biological samples in the study clinic. Baseline sampling commenced within the first days of the beginning of the spring semester and lasted for one week (i.e. from January 31, 2017, to February 5, 2017). During this period, all eligible participants who were willing to participate were asked to complete a survey form which contained questions about their baseline health conditions, vaccination history and other relevant social and behavioral information as shown in Table 2.

	Baseline 1	Baseline 2	Case	Contact
Surveys				
Demographic variables	+	-	-	-
Ten frequent contacts	+	+	-	-
Perceived Stress Scale	+	+	+	+
Smoking History	+	+	+	+
Alcohol Intake	+	+	+	+
Sleep Duration	+	+	+	+
[a]BMI assessment	+	+	-	-
Biologic Samples				
Venous Blood for Serology	+	+	+	+
PAXgene blood	+	+	+	+
Combined Nasal and throat swabs	1 pair	1 pair	2 pairs	2 pairs
Fomite swab	-	-	+	-
Exhaled breath sample	-	-	+	-
Four frequent contacts	-	-	+	+
Hours spent in room	+	+	-	-

[a] Body Mass Index; Measurements were only taken in the second baseline if subjects had not participated in the first.
+ When surveys were conducted
- When surveys and procedures were not carried out

We asked about questions that relate to their baseline social characteristics such as alcohol consumption pattern, sleep pattern, and cigarette smoking habits (Appendix A). We also assessed their baseline perceived stress and physical activity levels using validated questionnaires (55,56) also shown in the Appendix A. Each participant was asked to select from a list containing all eligible participants, up to 10 students with whom they frequently interact. All the participants were educated about the symptoms of ARI and asked to report to the study clinic by text, call or via e-mail as soon as they noticed any of the ARI symptoms during the semester. In addition to survey sampling, we obtained hair and/or nail specimens, venous blood specimens,

anterior nasal and oropharyngeal swab specimens from all participants at baseline.

(See specimen collection protocol below).

Case Evaluation

We defined as case, any subject whose combined nasal and oropharyngeal swab

specimens contained a viral ARI pathogen using a qRT-PCR analysis. We classified

cases as either primary or probable secondary. Primary cases were further classified

into the self-reported case and surveillance-positive primary cases (Figure 1, Table

3a).

Figure 1. Biological samples obtained and the order of their collection in cases and contacts.
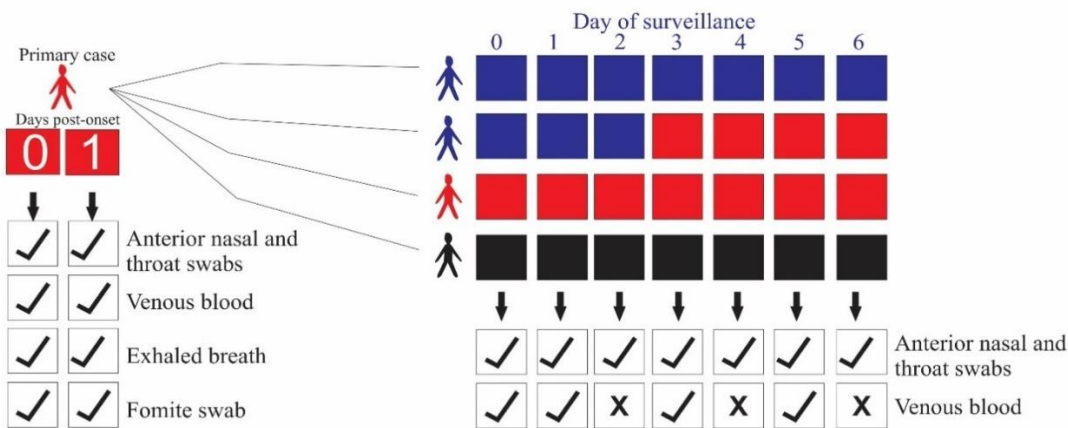
We attempted to recruit 4 contacts for each positive primary case. Each recruited contact was evaluated for 7 days and the biological samples were obtained from them in the order shown.
The blue colored boxes indicate negative contacts visit while red indicates a positive contact visit with a matching pathogen with that of the primary case. Black colored boxes indicate contacts who were positive for a pathogen different from that of the primary case.

We defined as a self-reported case, any subject with an illness that constituted any

upper respiratory symptom and/or a cough and/or a sore throat and that was reported

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to the clinic within 48 hours of onset. Surveillance-positive primary cases were those who were originally recruited as contacts but whose infector could not be determined by contact network pathogen matching method.

	Notes
Primary Cases	Cases who can be regarded as the source of infection within a *contact network group or cluster. They were the first in the cluster to be detected with the pathogens of interest in a given 7-day period.
Self-reported primary cases	These are the subjects that reported their illness directly to the study clinic, whose infections were qRT-PCR confirmed and could not be determined to be from any other subject in their cluster in the 7days before onset.
Surveillance-positive primary cases	Subjects under surveillance who tested positive, whose infector could not be determined either through epidemiological pathogen matching.
Probable Secondary Cases **(for p**hylogenetic analysis)	These include: i. Self-reported subjects whose infector(s) can be inferred to have come from a member of a cluster via epidemiological pathogen matching ii. Surveillance-positive contacts with a matching infection with nominating case. iii. Surveillance-positive contacts with infections that was traced to another member of the cohort in the 7 days prior to onset. i.e. contacts who had the same infection with another participant in the same social network group within 7 days of illness in the first.
Surveillance negative contacts	Nominated contacts that did not test positive while under surveillance.

* A contact network group refers to individuals who either nominate each other as contacts or live on the same floor in the dormitory.

Only participants in the main cohort were eligible to be evaluated as self-reported cases. Self-reported cases were evaluated on up to 2 consecutive days in the clinic depending on the result of the tests carried out on the swab specimens obtained from them on the first day of clinic evaluation. Only those whose specimens were positive were invited for re-evaluation on the second day of case testing. At each of those visits, in addition to updating their social characteristics, we obtained anterior nasal and oropharyngeal swab specimens, fomite (cellphone) swab specimens, venous blood, and exhaled breath specimens. Nose, oropharyngeal, fomite, and exhaled breath specimens facilitate examination of viral shedding and respiratory microbiome predictors in models of contagiousness and susceptibility. Self-reported cases were asked to identify up to 4 people in the target cohort with whom they interacted in the preceding 24 hours. Their swab specimens were immediately processed to identify viral pathogens. A follow-up questionnaire was sent to the cases on the 3rd day asking them to update their symptoms and to report any new contact they might have interacted with in the previous 24 hours.

Contact surveillance and evaluation

All the students in the overall eligible group who were selected as close contacts by the cases were eligible to be enrolled in the contact arm of the study. To enroll these contacts, we sent recruitment e-mails to their university e-mail addresses and handed recruitment flyers to the corresponding cases. To be able to describe pre-infectious phenotype, it was imperative to identify and enroll exposed contacts before they became symptomatic. We hence categorized contact subjects for effective pre-infectious analysis as described in Table 2b.

	Notes
Surveillance positive contacts	
Positive on the first day of contact visit	Subjects who were positive on the first day of their surveillance
Positive on day 2+	Subjects who were negative on the first day of their surveillance but later became positive during surveillance.
Surveillance negative contacts	Nominated contacts that did not test positive while under surveillance.

Given the serial interval of about 2.6 days for influenza (31,35), we only enrolled

contacts who presented to the clinic within 3 days of being named by a case, on the

premise that they would have already become symptomatic by the 4th day. Enrolled

contacts were evaluated at the clinic every day for 7 days during which they were

asked to complete survey questions which asked about their contacts as well as

questions that relate to their health status (Table 1). We obtained nasal and

oropharyngeal swabs on all days of clinic visits, but venous blood samples were only

collected on days 1, 2, 4 and 6 of study clinic visits as shown in Figure 1.

We alternated the blood collection routine to increases the likelihood of obtaining

pre-infectious blood samples which we presume to be essential for the identification

of pre-infectious genetic markers. Their nasal and oropharyngeal swab specimens

were processed at the laboratory using qPCR and tested for the presence of 44

infectious agents. Contacts who tested positive during the surveillance period were

referred to as *surveillance positive contacts*. If they were tested positive for the same ARI virus that the case who nominated them had, then they were referred to as *probable secondary cases* and were evaluated using same procedures as we did the self-reported cases while contacts that on the other hand remained negative throughout the seven daily visits were referred to as *surveillance negative contacts.* The remaining subject categorization is presented in Table 3a. For the pre-infectious analysis, we divided contacts into those who were positive on the first day of surveillance, those who were negative on the first day of surveillance but later became positive and those who were never positive throughout surveillance (Figure 3b).

We sent weekly emails to the target cohort to inquire about illnesses and to encourage those who were sick to come to the study clinic for screening and potential evaluation as soon as symptoms arise. Undergraduate research assistants were assigned as liaisons to each member of the cohort. Liaisons maintained communication with study participants throughout the course of the study via the communication medium of choice of each participant. Contacts of cases were contacted by email and brochures that were given to the cases to distribute (if they agreed), and by liaison outreach.

Specimen Collection Protocol

We collected different numbers and types of specimens at different clinic visits. The number and type of sampling done in the case- and contact-visits are shown in Table 1 and Figure 1. We did an initial baseline sample collection between

January 31, 2017, and February 5, 2017. At the baseline, we obtained a paired nasal

swab and oropharyngeal swab from the participants. We sampled the anterior part of

one nostril by rotating the swab applicator 3 times and then placing it inside a vial

containing 1 mL of universal viral transport medium (UVT) (Becton, Dickson and

Company, Sparks, Maryland USA). An oropharyngeal swab was obtained by

sampling the posterior throat and placing the swab into the vial containing the

anterior nasal swab to make a combined nasal and oropharyngeal specimen. At the

case and contact evaluation visits, we obtained 2 pairs of nasal and oropharyngeal

swabs thus producing 2 pairs of combined nasal and oropharyngeal specimens. All

samplings were done using nylon-tipped flexible plastic-shafted applicators (Becton,

Dickson and Company, Sparks, Maryland USA). The combined nasal and

oropharyngeal swabs have been shown to be at least as sensitive as a single

nasopharyngeal swab (57).

The specimens were tested using TaqMan® Array Card (TAC; Thermo Fisher

Scientific, Waltham, MA, USA) which is designed to rapidly detect up to 44 different

pathogens in a biological specimen. this enabled early confirmation of cases among

participants, aiding efficient epidemiologic contact investigation and surveillance of

likely transmission networks between friends and roommates with whom substantial

contact occurs.

We collected about 17.5mLs of venous blood from each participant at

baseline, 15mls of that was collected into a plain blood tube with no anticoagulant

while about 2.5mls of blood was collected into a PAXgene RNA tube (PreAnalytiX

GmbH, Hombrechtikon, Switzerland). We also collected nail clippings and samples of hair strands. Collected blood specimens facilitate investigation of transcriptome and immunome signatures, while hair and nail specimens were archived to enable subsequent assay for cortisol as a stress biomarker as an additional measure of stress, a known marker of infection susceptibility (58).

A second baseline sampling was done after spring break, between March 27, 2017, and April 3, 2017, and all the initial baseline procedures were repeated. At each case visit, we swabbed the cellphones of the subjects using a swab stick dipped in UVT solution. The symptom card presented to the subjects to rate the severity of their symptoms at each of the clinic visit is described in the Appendix A. The exhaled breath was collected using a Gesundheit-II, as previously described (59), and archived for future analysis.

Data and Statistical Analysis

All data were cleaned and analyzed using R software. (60). We defined as upper respiratory, symptoms of sneezing, sore throat, runny nose, stuffy nose or an earache. A lower respiratory symptom was described as any of shortness of breath, cough or chest tightness while systemic symptom was any of malaise, headache, muscle and joint ache, chills-rigors-fever, nausea, vomiting, diarrhea. We calculated scores for each symptom category by summing the scores reported for each symptom in that category.

To identify subjects with more severe illnesses, we defined a term called mild-to-moderate acute respiratory infections (MMARI) which depends on the PCR cycle threshold (CT) value, the subject's reported symptoms, the measured oral temperature at the time of visit and the persistence of the infection in the subject. The Ct value is the number of cycles required for the fluorescent signal to cross the threshold (i.e. exceeds background level). We defined as an MMARI, any case of a subject who fulfills any of the following : (a) tested qPCR positive with a CT value of less than 33 at least once in a consecutive series of visits regardless of other parameters, (b) tested qPCR positive with a CT of 33 or more on detected on at least 2 consecutive days, (c) had one of sore throat, cough, stuffy nose, runny nose, shortness of breath, or chest tightness in addition to having a CT value of at least 33, (d) whose measured oral temperature was at least 38 degrees in addition to having a CT value of at least 40.

The difference in means of the symptom scores between positive and negative self-reported cases was tested using the Student's t-test. While the difference in the proportion of the subjects with MMARI was tested using the Chi-square test. Statistical significance was assessed using two-tailed tests with an error level of 0.05. The statistical analysis was conducted using the base statistical package of R software. The contact network analysis was done using the "igraph" R package (61).

Network Analysis

We aggregated all contacts nominated by each subject during the study to form a list and then used the list as the subject's contact network. We analyzed basic contact network properties, including degree and edge density in order to assess the

overall connectivity of the network. The degree is the total number of contacts reported by each student during the study while edge density is the ratio of the number of links present in the network and the maximum number of links possible(62).

Ethics Statement

This study was approved by the Institutional Review Boards (IRB) of the University of Maryland and the Department of Navy Human Research Protections Office. Electronic informed consent was obtained from all participants through an electronic signature applied on an online form sent to each participant via e-mail at the time of baseline assessment. An in-person informed consent was obtained at the time of baseline specimen collection. Repeat in-person consenting was done each time a participant was to be enrolled in either the case or contact arm of the study. All consent forms for this study were signed electronically.

Chapter 4: Results

Characteristics of the subjects

Our study population comprised of college dormitory students; mostly first-year students (91.2%); 72% females and aged between 18 to 19 years. The descriptive statistics of the demographic, health behavior, health status, and psychosocial variables of the study population are shown in Tables 4a and 4b.

Table 4a. Demographic covariates of viral ARI pathogen detection in all subjects and in contacts.

Variable	All subjects N=68	Surveillance-negative contacts N=16	Surveillance-positive contacts N=24
Mean age in years ± SD	18.21 ± 0.41	18.07 ± 0.27	18.17 ± 0.38
Female (%)	49 (72)	11(68.8)	20 (83.3)
Race (%)			
White	29 (42.6)	8 (50)	12 (50)
Black/AA	16 (23.5)	2 (12.5)	6 (25)
Asian Indian	6 (8.8)	1 (6.3)	3 (12.5)
Chinese	4 (5.8)	1 (6.3)	0
Filipino	3 (4.4)	2 (12.5)	0
Korean	3 (4.4)	0	0
Vietnamese	2 (2.9)	0	1 (4.2)
Other Asian	4 (5.8)	0	1 (4.2)
Unknown	1 (1.5)	0	0
Multi-racial	1 (1.5)	0	1 (4.2)
Academic Year, N (%)			
First year	62 (91.2)	16 (100)	23 (95.8)
BMI kg/m^2 ± SD	24.54 ± 4.4	22.63 ± 3.3	23.12 ± 3.2
Smoker, current (%)	4 (5.89)	1 (6.25)	3 (12.5)
Number of hours spent in room	13.03 ± 2.98	13.72 ± 1.42	12.25 ± 3.55

Abbreviation: SD, Standard deviation

We compared the study participant characteristics to that of the overall student population, and to students residing in other university's on-campus residence halls.

There was a higher proportion of females in our study sample (72%) than in the

overall student population (46.6%). However, race and ethnicity proportions in our

study population (White: 42.6%) were close to those of the overall student population

(White; 42%).

Table 4b. Demographic covariates of viral ARI pathogen detection in all subjects and in contacts. (Continued)

Variable	All subjects N=68	Surveillance-negative contacts N=16	Surveillance-positive contacts N=24
Asthma, self-reported (%)	8 (11.8)	2 (12.5)	3 (12.5)
Ever received influenza vaccination (%)	49 (80%)	10 (62.5)	20 (83.3)
Influenza vaccination previous season (%)	34 (55.7%)	10 (62.5)	13 (54.2)
Influenza vaccination current season (%)	26 (42.3%)	6 (37.5)	9 (37.5)
Number of vaccinations	2.7 ± 0.7	2.8 ± 0.4	2.6 ± 0.75
Ever smoked cigarette (%)	3 (4.4)	1 (6.25)	2 (8.3)
*Perceived Stress Score	20.13 ± 4.13	19.00 ± 6.47	20.38 ± 3.29
**Physical Activity, median (IQR)	5,437.5 (3255.5-7917)	5,5345 (3,355-7,776)	5,5256.8 (3455.5-7576)
Sleep duration – hours (Weekdays)	7.52 ± 1.2	7.73 ± 1.2	7.66 ± 1.4
Sleep duration - hours (Weekends)	9.14 ± 1.4	9.6 ± 1.3	9.04 ± 1.49
Alcohol (Drinking times per week)	1.05 ± 1.82	1.87 ± 3.1	0.86 ± 0.92
Alcohol (Drink number per episode)	2.28 ± 2.44	2.7 ± 2.9	1.96 ± 2.1

*Perceived Stress Score: Mildly stressed; 0-13, Moderately stressed; 14-27, Severely Stressed; 28-40.
**Metabolic Equivalent-minutes: The ratio of work metabolic rate to the resting metabolic rate. 1 MET is 1kcal/kg/hour

Study participation and enrollment

We collected baseline survey data from 75 subjects out of a total of 146 eligible
participants. Of the 75 subjects, 9.3% (N=7) did not provide any baseline biological
sample and hence were excluded from all analyses while the remaining 68 from
whom we obtained complete data were retained in the analyses. 73.5% (N=50) of
these provided baseline biological samples at the beginning of the semester between
January 31, 2017, and February 5, 2017, and 88.2% (N=60) provided biological
samples mid-semester between March 27, 2017, and April 3, 2017, i.e. during the
second baseline collection period. Only 67.6% (N=46) of all subjects provided
samples at both baseline sampling period (Figure 2). We screened 59 episodes of self-
reported illnesses reported by 38 subjects some of whom reported more than one
episode during the study period. As shown in Figure 2, of these 59 illness episodes 24
(44.4%) were positive for viral ARI pathogens as evaluated by the qRT-PCR test that
was performed within 24 hours of presentation and was accounted for by 20 of the 38
subjects (52.6%).

Figure 2. Flowchart showing the number of visits to different kinds that occurred
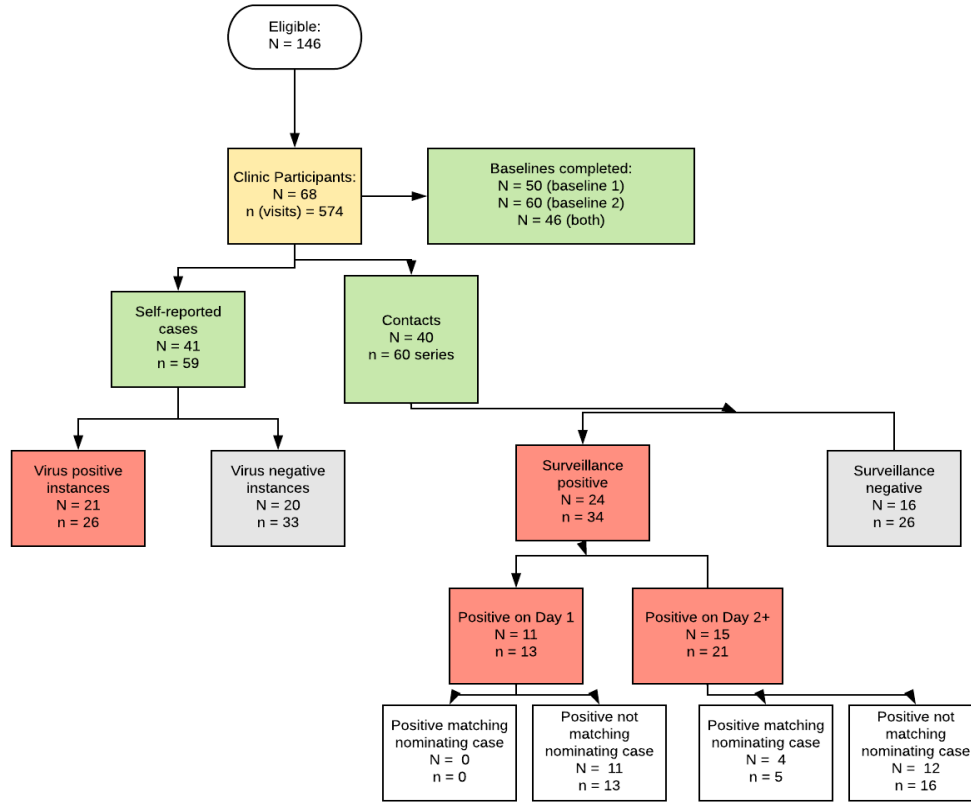

N, unique subjects. n, number of instances of that visit.

Though we attempted to enroll for surveillance, 4 subjects for each of the 59
confirmed episodes of illness that the cases presented with, we were only able to
enroll 60 instances in total. Thus, we enrolled 1.1 contacts per case. Figure 3 displays
the temporal distribution of the positive self-reported cases during the study period
and when different pathogens were detected.

There was a midsemester, spring break between March 19 and March 30, hence no
student was tested during that period.

Figure 3. Temporal distribution of the self-reported cases.
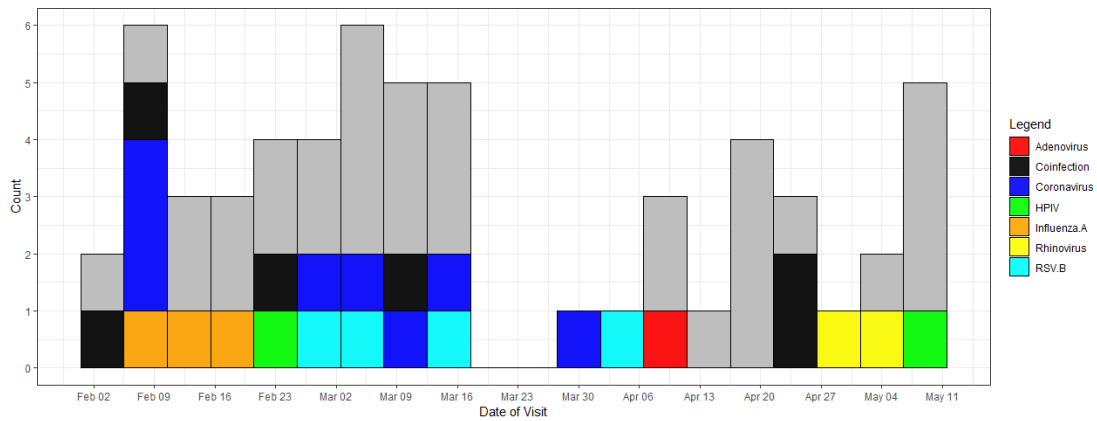
Abbreviation. HPIV: Human Parainfluenza Virus
Coinfections: 1 subject had 3 viral coinfections, 5 cases were co-infected with 2 pathogens

Pathogens detected

Figure 4. Viral pathogens detected in the cohort during the semester
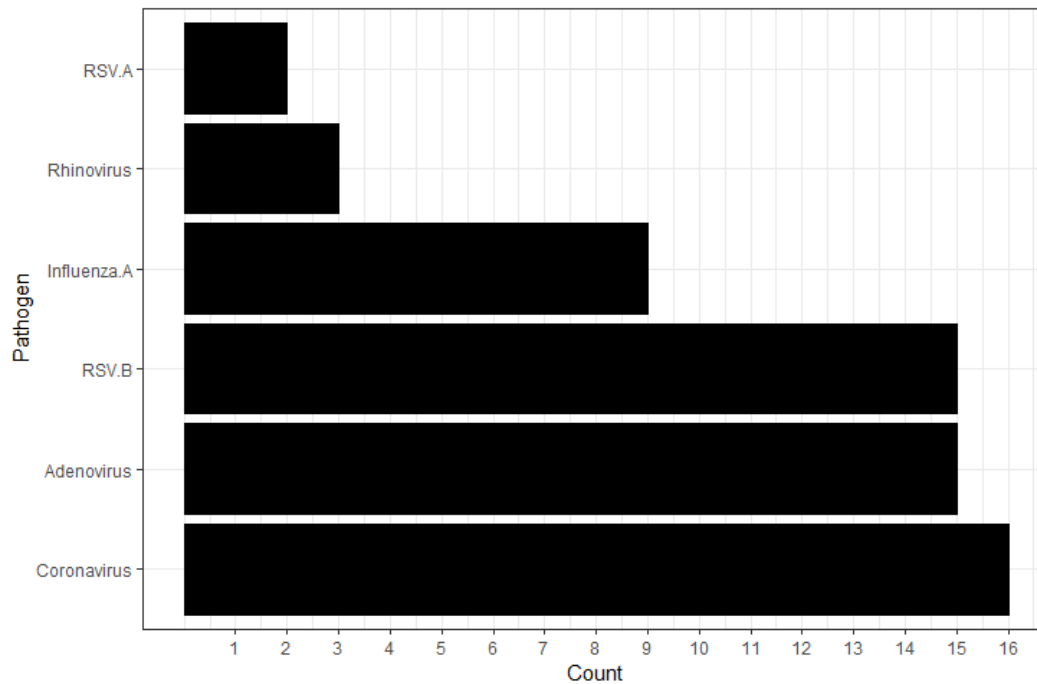
There were 72 laboratory-confirmed episodes of infections in the cohort. 9 episodes of Influenza A (H3N2) illnesses were detected. 15 episodes of Respiratory Syncytial Virus (RSV) illnesses were detected in the cohort. Adenovirus infection was detected

in 15 episodes of illness while rhinovirus was only detected in 3 illness episodes.

Figure 4 shows the number of episodes of various pathogens that were detected.

Of the 60 contacts surveillances that were carried out, we did not detect any pathogen

in 26 throughout their surveillance period while there were 34 surveillance positive

contact visits. Out of those 34 episodes, 13 (38%) were already positive on the first

day of contact surveillance and 21 were tested positive on subsequent follow-up

visits. Of the surveillance positive contacts, there were 5 whose pathogens matched

those of the nominating cases. By this metric, the secondary attack rate was 8.3%.

Study network and contact surveillances

Contacts and cases were divided into categories based on the relevance of their

contribution to transmission network analysis and pre-infectious analyses as

described in Tables 2a and 2b. The results of these are summarized in Tables 5a and

5b.

Table 5a. Classification of Contacts for Biomarker Analysis.

	All ARI	Influenza A
Positive on the first day of contact visit	11 (13) *	1 (1)
Positive on day 2+	15 (21)	3 (3)
Surveillance negative	16	36

* The number in parenthesis is the total number of instances observed while the number outside the parenthesis is the number of unique subjects observed

Most of the contacts who were not positive on the first day of surveillance tested PCR

positive on the 3rd day of surveillance (Figure 5). The mean CT value on the first day

of surveillance in surveillance-positive contacts was 33.5. Only 4 (25%) of the

surveillance-positive contacts had sustained-qPCR-detectable (i.e. CT value < 38

cycles) infection on days 1 and 2 following the first day of detection (Figure 6).

Table 5b. Classification of cases and contacts for transmission analysis.

	All ARI	Influenza A
Primary cases		
Self-reported cases	46	5
Surveillance positive primary	16	3
Probable Secondary Cases	5	1
Surveillance negative	16	36

Figure 5 Showing the number of contacts who tested positive and when they did during surveillance.
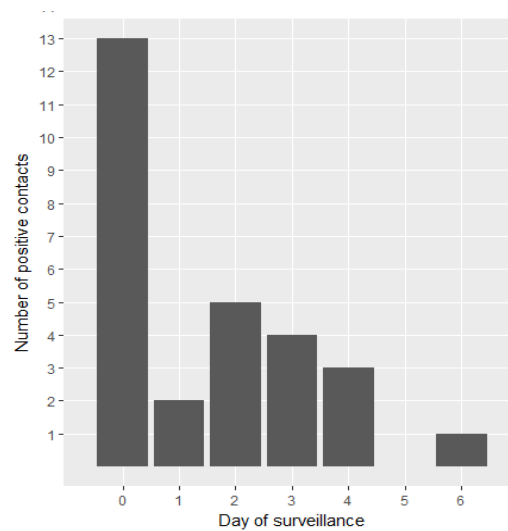
0 is the first day of surveillance.

Figure 6. Summary of CT values for the surveillance-positive cases from the first day viral pathogens were detected in them.
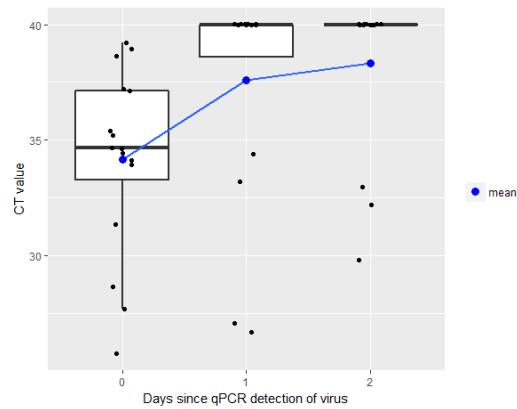
Abbreviation. CT: Cycle threshold. The Ct value is the number of cycles required for the fluorescent signal to cross the threshold (i.e. exceed background level)

Symptom pattern of the study group

Symptom pattern and in positive self-reported cases is shown in figures 7a, 7b and Table 5.

Figure 7a. Summary of the reported symptom scores of all positive cases on the first day of their clinic visits
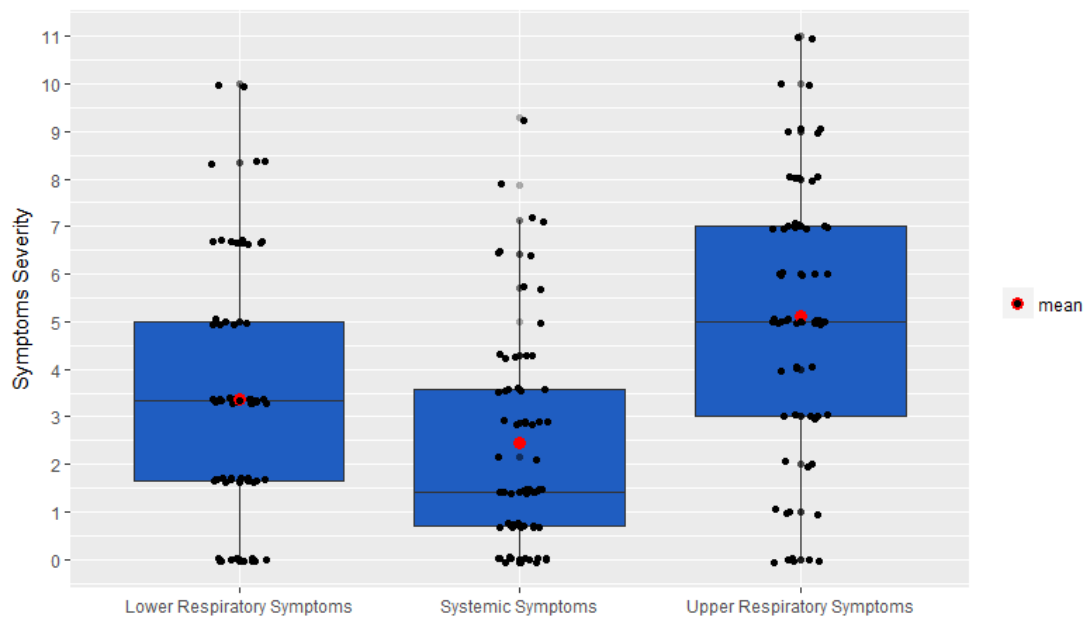
Upper Respiratory Symptoms: Sneezing, Sore throat, Runny nose, Stuffy nose or an Earache,
Lower Respiratory Symptoms: Cough, Shortness of breath, Chest tightness
Systemic Symptoms: Fever, Headache, Malaise, Joint and Muscle aches

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Figure 7b. Measured oral temperature in the detected ARI cases on the first day of their clinic visit.
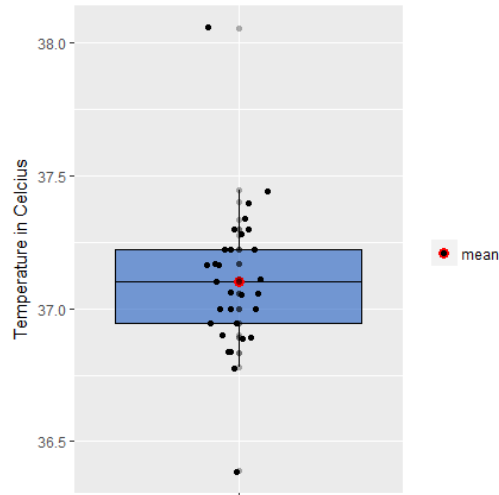
Most self-reported cases were only mildly symptomatic. The mean measured oral

temperature was 37.3'Celcius. The most common symptoms reported in negative

self-reported cases were; stuffy nose, malaise, and sore throat. The distribution of the

self-reported symptom scores is summarized in Appendix B. As shown in Table 6a, a

statistically significant difference was observed in the reported scores for a headache,

sneezing and runny nose between the positive self-reported cases and the negative

self-reported cases. Table 6b summarizes the reported symptoms for the contacts who

were tested.

Table 6a. Summary of symptom scores for the self-reported cases of ARI.

	Viral infection detected N=26		No viral infection detected N=33		p value
	Mean	Median	Mean	Median	
Measured Oral Temperature	37.3	37.1	36.8	36.8	0.35
Upper Respiratory Symptoms	**6.3**	**6**	**5.03**	**5**	**0.03***
Runny Nose	**1.6**	**2**	**1.1**	**1**	**0.02***
Stuffy Nose	1.6	2	1.4	1	0.33
Earache	0.3	0	0.6	0	0.14
Sneeze	**1**	**1**	**0.6**	**0**	**0.03***
Sore throat	1.8	2	1.4	1.5	0.09
Lower Respiratory Symptoms	2.3	2	2.15	2	0.65
Cough	1.5	2	1.4	2	0.56
Shortness of Breath	0.5	0	0.5	0	0.69
Chest Tightness	0.3	0	0.3	0	0.94
Systemic Symptoms	3.3	2	4.5	4	0.17
Headache	**0.6**	**0**	**1.3**	**1**	**0.01***
Fever, chills	0.8	0	0.6	0	0.4
Malaise	1.2	1	1.5	2	0.2
Muscle Joint Ache	0.5	0	0.8	0	0.26
Others					
Diarrhea	0	0	0.03	0	0.33
Nausea	0.2	0	0.3	0	0.44
Vomit	0.1	0	0	0	0.16
MMARI, N (%)	26 (100)		-		

* p-value < 0.05 Abbreviation, MMARI, Mild-to-Moderate Acute Respiratory Infection

To qualify as an MMARI, a case must have; (a) been tested qPCR positive with a CT value < 33 at least once during the series of consecutive visits regardless of other parameters, (b) been tested qPCR positive on 2 consecutive days with CT value ≥ 33, (c) had one of sore throat, cough, stuffy nose, runny nose, shortness of breath, or chest tightness in addition to having a CT value ≥ 33 during the visit, (d) tested positive with CT value ≥ 33 and have a measured oral temperature was ≥ 38 degrees.

Table 6b. Showing the summary of symptom scores for the contacts tested for ARI

|	Positive on Day 1 (N=13)	Positive on Day 2+ (N=21)	Never positive (N=16)
	Mean	Mean	Mean
Measured Oral Temperature	36.87	36.84	36.8
Upper Respiratory Symptoms	2.39	1.89	1.02
Runny Nose	0.46	0.53	0.27
Stuffy Nose	0.77	0.47	0.29
Earache	0.08	0.12	0.04
Sneeze	0.3	0.24	0.18
Sore throat	0.77	0.53	0.23
Lower Respiratory Symptoms	0.92	0.59	0.58
Cough	0.85	0.59	0.37
Shortness of Breath	0.08	0	0.19
Chest Tightness	0	0	0.02
Systemic Symptoms	1.08	0.54	0.47
Headache	0.38	0.18	0.13
Fever, chills	0	0	0
Malaise	0.38	0.24	0.27
Muscle Joint Ache	0.23	0.12	0.04
Others			
Diarrhea	0	0	0.03
Nausea	0.08	0	0.01
Vomit	0	0	0
MMARI, N (%)	8 (61.5)	4 (19)	-

Abbreviation, MMARI, Mild-to-Moderate Acute Respiratory Infection
To qualify as an MMARI, a case must have; (a) been tested qPCR positive with a CT value < 33 at least once during the series of consecutive visits regardless of other parameters, (b) been tested qPCR positive on 2 consecutive days with CT value ≥ 33, (c) had one of sore throat, cough, stuffy nose, runny nose, shortness of breath, or chest tightness in addition to having a CT value ≥ 33 during the visit or (d) tested positive with CT value ≥ 33 and have a measured oral temperature of ≥ 38 degrees.

16 episodes of surveillance positive contacts were identified in the cohort contributed by 15 subjects (Tables 2a and 2b). All the surveillance positive contacts reported one or more symptoms on the day they tested positive. The mean symptom score was higher for upper respiratory, lower respiratory and systemic systems on day 2 following the detection of the viral pathogen. On the first day of viral pathogen detection, only 25% (4 of 16) of the surveillance positive contacts had measured oral temperature that was greater than 37.0° Celsius. The mean measured oral temperature in the surveillance negative contacts was 36.8° Celsius. Summaries of the symptom score and temperature progression before and after qRT-PCR detection of viral pathogens in the surveillance positive contacts are shown in Figures 8a and 8b.

Figure 8a. Symptom progression in the positive contacts before and after qRT-PCR detection of viral pathogen.
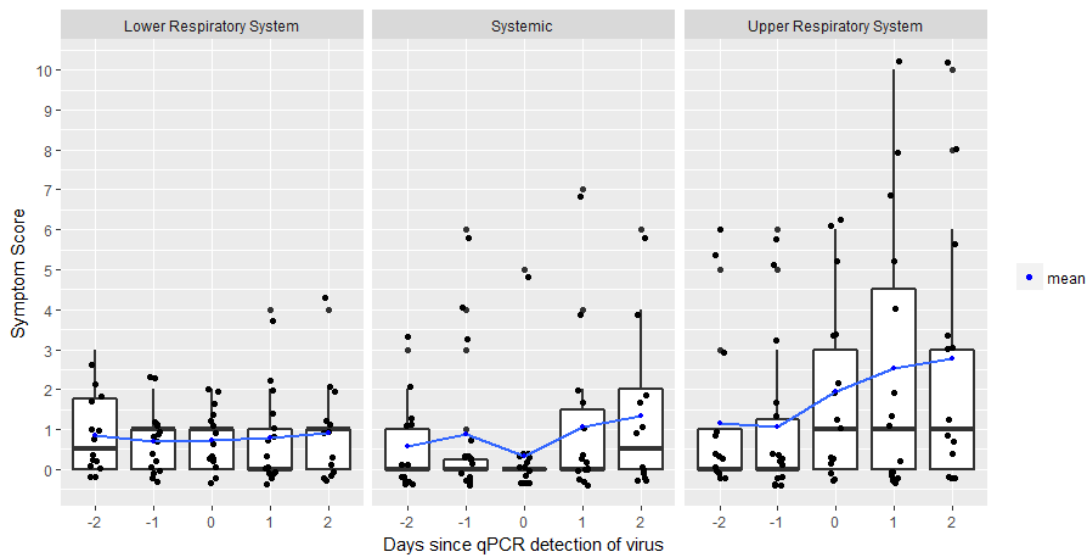
Upper Respiratory Symptoms: Sneezing, Sore throat, Runny nose, Stuffy nose or an Earache,
Lower Respiratory Symptoms: Cough, Shortness of breath, Chest tightness
Systemic Symptoms: Fever, Headache, Malaise, Joint and Muscle aches

Figure 8b. Oral temperature progression in the positive contacts before and after qRT-PCR detection of viral pathogens
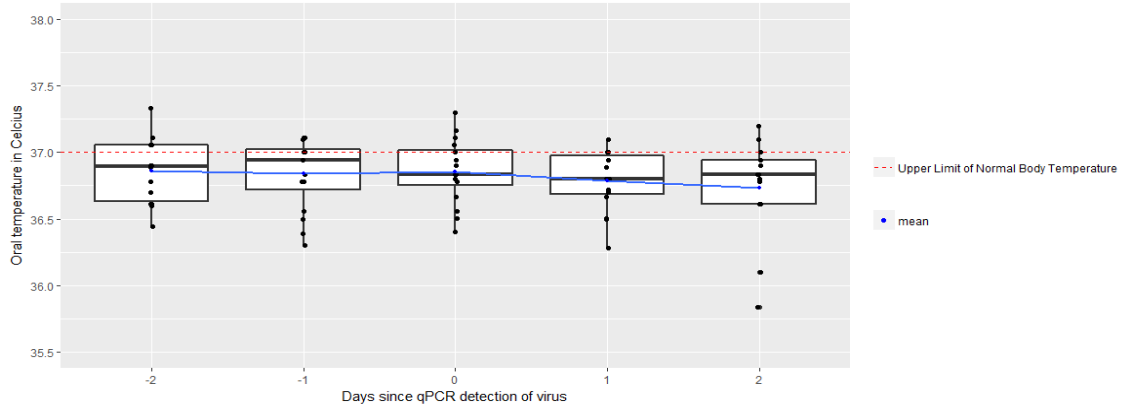
Network Analysis

The link density i.e. the ratio between the total number of connections between the subjects and the total number of possible connections is 18.5%. Figure 9a shows the basic contact network structure and measurements of the subjects including the mean degrees, the density of the network. Figures 9b and 9c illustrate the individuals who tested positive for Influenza A and RSV-B and their respective positions in the network.

Figure 9a. Contact network structure of the participating subjects in study cohort based on 24-hour contact selection.
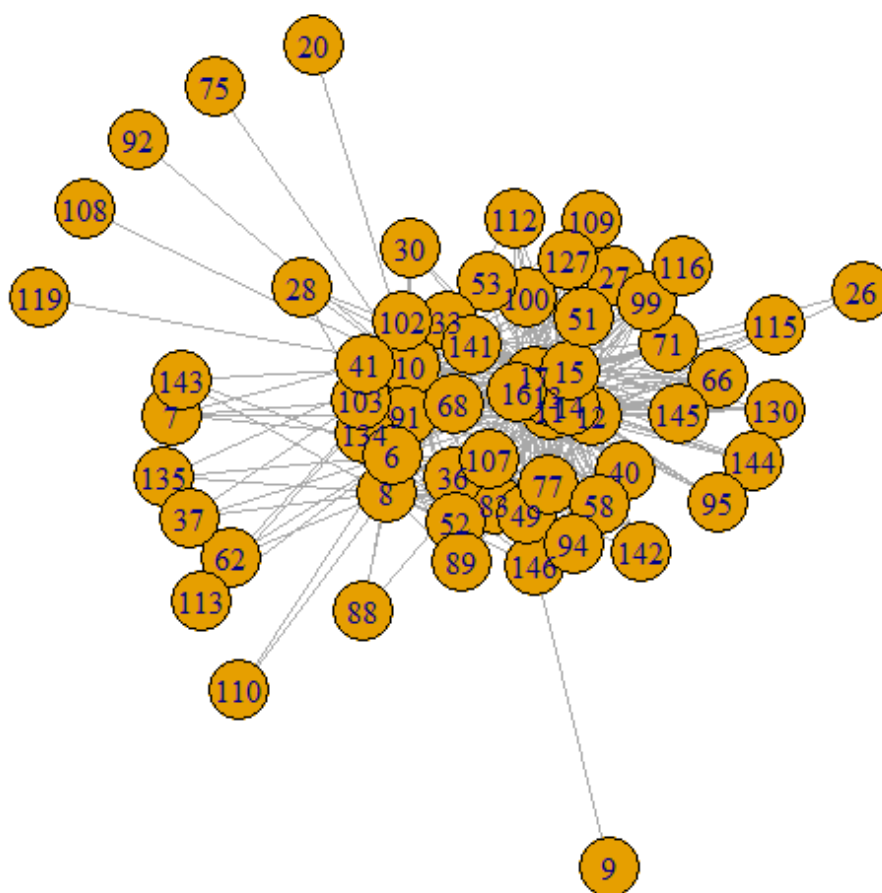
The golden circles (nodes) represent the subjects while the black lines represent the links between them. Degree is the total number of contacts reported by each student during the study while the edge density is the ratio of the number of links present in the network and the maximum number of links possible.
Average degree: 11.5
Edge density: 18.6%

Figure 9b. Contact network structure of the participating subjects showing individuals who tested for Influenza A
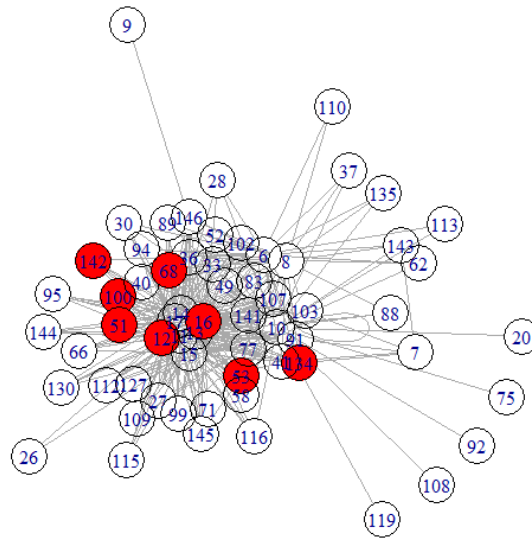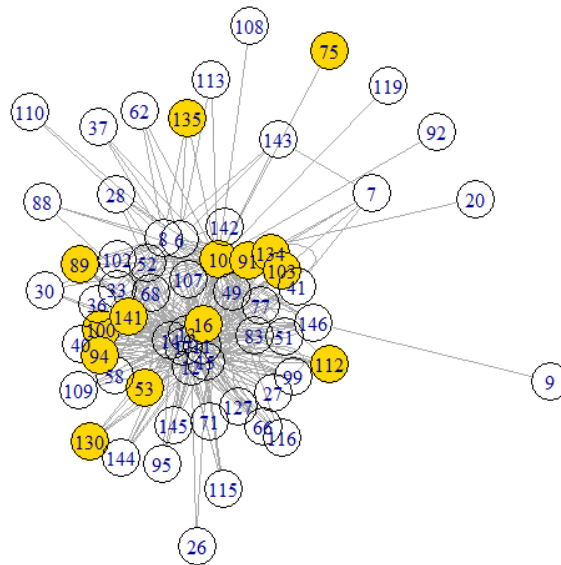

Figure 9c. Contact network structure of the participating subjects showing individuals who tested for RSV-B

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Chapter 5: Discussion

Here we have described a college resident community surveillance study where we prospectively monitored a select group of students during a semester to identify and characterize cases of ARI and their transmissions among their contacts. Using self-reported contact nomination system, the design of the study provided an opportunity to monitor an extended contact network of the subjects with ARI beyond their immediate living location (i.e. their rooms). This distinctiveness is an improvement over household ARI studies, which are generally limited given that they often fail to characterize or identify transmissions that occur outside households. This meant that we could potentially account for transmissions that occur in non-household settings such as in the library, study rooms, classes, and diners. The prospective nature of the study meant that we could obtain serially, biological specimens from ARI cases from the day of self-reporting their illness and for 7 days in the exposed contacts till they become ill thus providing a great amount of data about the natural course of illness in ARI cases and the pre-infectious and convalescent phenotype and genotype in the surveillance-positive cases. Other specimens that we obtained: nose, oropharyngeal, fomite, and exhaled breath specimens help facilitate examination of viral shedding and respiratory microbiome predictors in models of contagiousness and susceptibility. Our study eligibility criteria reflected our goal to maximize participation rates. We mainly targeted college students in a specific program with an academic focus on health on the presumption that they would be more willing to participate than other students in non-health-related programs. An additional advantage of enrolling this group of students is that the dormitory where the majority live was adjacent (about

50ft away) to the study clinic and the laboratories of the School of Public Health. The study was set out to enroll 4 close contacts for each of the self-reported cases hence we restricted the eligible members of our cohort to residents of certain floors in the dormitory where most LLC members live (Table 1). We believed 4 contacts were reasonably easier for the participants to recall and that number is logistically feasible to follow up. Meanwhile, as our target group was a tightly-knit community where many of the cases had the same contacts, we could not achieve our goal of enrolling 4 close contacts per case.

For the enrolled exposed contacts whom we followed-up for 7 days, we alternated the order of venous blood sampling for the following reasons (Figure 1): (1) to increase the chances of drawing a day-minus-1 blood specimen (i.e. blood obtained a day before qPCR detection of viral particles) and (2) to increase the toleration of blood draws among the participants. However, we discovered upon analysis (Figure 5), that most of the contacts who became positive, developed illness within the first 5 days of surveillance. This suggests that the most important days for evaluating contacts and drawing blood specimens are the first 5 days of enrollment and that by alternating blood draws we inadvertently obtained a fewer number of day-minus-1 blood specimens than the numbers we could have obtained if we had drawn blood daily on the first 4 days of surveillance. This observation is in keeping with reports from experimental influenza and ARI studies which identified that following inoculation, most infected subjects exhibited signs of infection within the first Also, a systematic review which examined the serial intervals in 18 influenza household studies, reported that median serial intervals in those studies ranged from 2.8 to 4.0 (34).

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We found that most of the enrolled contacts were already positive at the time of enrollment implying that most transmissions and onset of disease had occurred in the contacts prior to the commencement of the surveillance. Though we encouraged the study participants to contact us as soon as they noticed symptoms, it was impossible to objectively ascertain the onset of illness in the self-reported cases. Furthermore, in the exit survey that was conducted at the study completion, many participants reported that they were too busy to report to the clinic when they were sick. We think that if we made evaluation less burdensome e.g. by obtaining biological specimens from the participants at their convenience in their rooms, we might be able to enroll cases and contacts more promptly in the course of their illness and perhaps enroll more contacts before they test positive.

The secondary attack rate of ARI in our target population was 8.3%. Despite our conviction that we did not observe some ARI transmissions due to some of the reasons mentioned earlier, the attack rate in our study is comparable to what some household studies have reported which ranges from 3% to 36% (34). Some studies have observed differences in attack rates of ARI in households where the index case was a child with some reporting higher attack rates (40,41). Given the relative homogeneity of our study population with a mean age of 18.21+/-0.41, it might be vital to investigate the transmission dynamics in a different population in the college environment. Perhaps, by expanding the study target group to capture the staff and faculty that interact with children such as those at the center that provides daycare services for pre-school kids as well as the enrolling the kids themselves, we might be able to characterize the transmission pattern in this important group.

Most of the qRT-PCR confirmed self-reported cases of ARI observed were only mildly symptomatic and so were the surveillance positive contacts. We assume that the more serious cases of ARI illnesses present to the University Health Center and/or remain confined to their dormitory rooms. Also, most of the surveillance positive contacts (75%) were only transiently qRT-PCR positive (i.e. we detected a viral pathogen by qPCR assessment once during surveillance but not on subsequent days) (Figure 6). While it is generally known that a substantial fraction of cases of ARIs and influenza are asymptomatic or associated with mild disease, it might still be essential to characterize more severe cases. If transmissibility is related to the severity of cases, then results from our study may not be generalizable to more severe cases. In a bid to identify the more severe cases of ARI in our cohort, we defined a term – MMARI. In future studies, it may be essential to carry out study recruitment exercise in the University Health Center in addition to the current design.

Self-report contact nomination was a very crucial part of our recruitment process for this study and its use allowed us to expound the connections within the network group in the study cohort. Given that most of the subjects (97%) in our cohort resided in the same dormitory, the identified contact network was tightly-knit with an edge density of 18.5%. In subsequent studies, when we expand to a larger cohort, we expect to see a network structure with well-defined clusters that may provide a suitable structure for the assessment of outbreak intervention strategies such as quarantine.

We collected details about the quality of contacts between subjects such as the duration and type of contacts, but those were not included in the network analyses in this paper (Appendix C). Though we asked participants to recall contacts made in the

past 24 hours, it may still be subject to recall bias. Hence, in subsequent studies, we will incorporate the use of a location-tracking phone application which will help identify when 2 or more users are in proximity and will help objectively quantify the duration of contacts between subjects enrolled in the study. Also, this may help to identify contacts promptly following exposure to an infected case and even allow us to identify a much larger number of potential contacts. In addition, it may help us to identify and describe the physical characteristics of specific locations where ARI transmission occurs. It will also be valuable in the future, to assess the correlation between the survey-collected contact nomination data and that collected via location tracking software.

The design and setting of the study meant that there was a need for extensive logistic efforts. Student liaisons had to be trained to help with recruitment and to keep reminding eligible students to contact the study staff when sick. An exit survey conducted towards the end of the study (baseline 2) to quantify unreported illness episodes revealed that 59% of those who did not report their illness failed to do so because they had forgotten. Also, the complexity and the large amount of the collected data meant that there was a need for extensive data management, cleaning, and analytic efforts.

This current study does not include data about the environmental and physical conditions of the student residential building, meanwhile, some studies have demonstrated the importance of indoor temperature and humidity in ARI transmission in animal models (63,64). Brundage et al showed a significantly higher incidence rate of ARIs among military trainees living in modern barracks with closed (recirculating)

ventilation buildings than in their counterparts living in the old barracks during a 47-month survey period (65). Other factors such as airflow (the speed of air currents flowing through indoor spaces) and ventilation (the degree of mixing between indoor and outdoor air) likely play important roles in virus infectivity and transmission of ARIs (64). One study showed a correlation between the probability of detecting airborne rhinovirus with the carbon dioxide (CO_2) content of the air which in turn related inversely to ventilation (66). Also, absent in this data are the results from the exhaled breath analysis. Another study which underscored the importance of examining the exhaled breath following the recovery of infectious influenza virus particles from the fine aerosols ≤5 micrometers of some subjects with confirmed influenza infections (67).

In conclusion, in this study, we have shown how ARIs transmission monitoring can be achieved in the student living and learning community and how it can provide a means of characterizing several relevant covariates. Though the study size was relatively small (N=68) we identified some probable secondary transmission. A follow-up phylogenetic analysis of the identified pathogens will help confirm transmissions. We believe this study design provides a unique opportunity to study the relative roles of many important covariates of ARI transmission. Upon expansion of the study to a larger cohort, incorporation of environmental monitoring measures improved contact network tracking and confirmation of transmission by phylogenetic analysis, we believe this design will provide a comprehensive data that will provide valuable insights regarding ARI transmission and control measures.

Appendices

APPENDIX A
<u>Symptoms Card</u>

0 = I have no symptoms, 1 = Just noticeable, 2= Its clearly bothersome from time to time, but it doesn't stop me from participating in activities, 3 = It's quite bothersome most or all of the time, and it stops me from participating in activities.

Runny Nose, Stuffy Nose, Sneezing, Sore Throat, Earache, Muscle (tiredness and fatigue), Headache, Muscle and/or joint ache, Sweats, fever, or chills, Nausea, Vomiting, Diarrhea, Chest tightness, Shortness of breath, Cough.

<u>International Physical Activity Questionnaire</u>

1. During the last 7 days, on how many days did you do <u>vigorous </u>physical activities like heavy lifting, digging, aerobics, or fast bicycling? Think about *only* those physical activities that you did for at least 10 minutes at a time.
2. Again, think *only* about those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do <u>moderate</u> physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.
3. During the last 7 days, on how many days did you <u>walk</u> for at least 10 minutes at a time? This includes walking at work and at home, walking to travel from place to place, and any other walking that you did solely for recreation, sport, exercise or leisure.
4. The last physical activity question is about the time you spent <u>sitting</u> on weekdays while at work, at home, while doing course work and during leisure time. This includes time spent sitting at a desk, visiting friends, reading traveling on a bus or sitting or lying down to watch television.
5. During the last 7 days, how much time in total did you usually spend *sitting* on a <u>week day</u>?

<u>Sleep and Alcohol History</u>

1. On how many days in the past week did you drink an alcoholic beverage?
2. On the days that you drank during the past week, how many drinks did you usually have each day? (Count as a drink a can or bottle of beer; a wine cooler, a glass of wine, a shot of liquor or a mixed drink or cocktail.)
3. On a typical weekday, how many hours per day do you spend sleeping? Include day and night.
4. On a typical weekend day, how many hours per day do you spend sleeping? Include day and night. Consider a typical week at school.

<u>Perceived Stress Scale</u>

0 = Never, 1= Almost Never, 2 Sometimes, 3= Fairly Often, 4 = Very Often

1. In the last month, how often have you been upset because of something that happened unexpectedly?
2. In the last month, how often have you felt that you were unable to control the important things in your life?
3. In the last month, how often have you felt nervous and "stressed"?
4. In the last month, how often have you felt confident about your ability to handle your personal problems?
5. In the last month, how often have you felt that things were going your way?
6. In the last month, how often have you found that you could not cope with all the things that you had to do?
7. In the last month, how often have you been able to control irritations in your life?
8. In the last month, how often have you felt that you were on top of things?
9. In the last month, how often have you been angered because of things that were outside of your control?
10. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?

Appendix B: Showing the distribution of the self-reported symptom severity scores (normalized) in all cases.
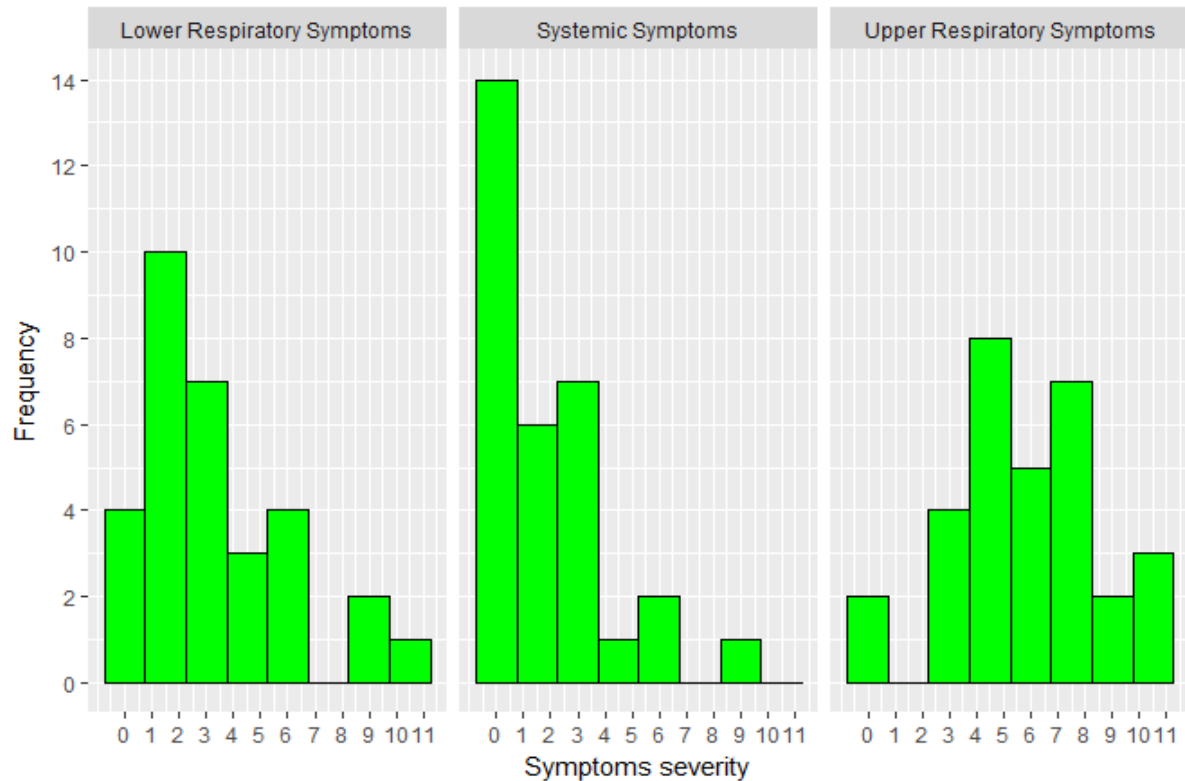
Normalization: The sum for each category was divided by the highest possible score then multiplied by 15 so the resulting scores are comparable across different categories

Appendix C. Showing the reported types of contact that occurred between subjects and the distribution of the duration of those types of contacts.
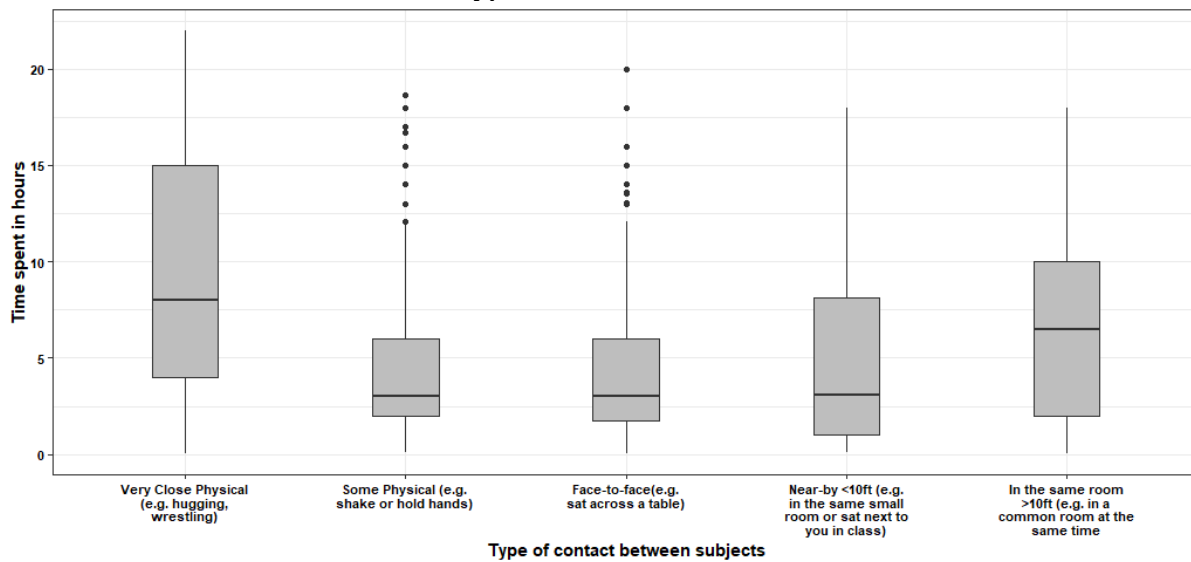
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