TraVerse:

A method of natural respiratory virus transmission from symptomatic children to healthy young adults

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Marie-jo Medina BS, MS, MSc

Department of Infection, Immunity and Inflammation

University of Leicester

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1 Abstract

A method of natural transmission that 'traverses' all identified common cold viruses was evaluated in Leicester, UK during 2012 to 2015. The viruses were: adenovirus, coronavirus, enterovirus, influenza, metapneumovirus, parainfluenza, rhinovirus, and RSV. Mechanisms of transmission assessed included large droplet, hand contact, fomite, and aerosol.

Aim: to augment current understanding of natural respiratory virus transmission using the TraVerse method.

Methods: TraVerse method of 30-minute interactions between symptomatic paediatric inpatients and healthy young adults in a fully functioning hospital ward. Swab and aerosol samples were tested using qualitative and quantitative PCR. Illness severities were evaluated using daily symptom diary cards.

Results: The overall RT-PCR positivity rates were 93% for children and 22% for adults. Rates of natural transmission, depending on the virus, were 4% - 24% by large droplet, 18% - 19% by hand contact, 50% - 100% by self-inoculation, and 8% - 33% by fomites. Aerosol transmission was not established. Adults did not shed flu, coronavirus, metapneumovirus, or parainfluenza; nevertheless, severe illness occurred in 26% whose child pairs had mono-infections of these viruses. When ill, only 7% of adults stayed home, and only 4% sought medical attention. Asymptomatic rhinovirus transmission occurred at a rate of 5%.

Conclusions: TraVerse was fit-for-purpose in elucidating natural, human-to-human respiratory virus transmission rates. Aerosol transmission may be the primary mechanism in the natural transmission of flu, coronavirus, metapneumovirus, and parainfluenza but was not appropriately evaluated because of funding constraints. Paediatric mouthing has relevance in nosocomial transmission. Healthy adults develop severe illness but do not generally stay home, take medications, or seek medical advice.

Recommendations: Sanitizing of paediatric patients' hands, use of masks to discourage face-touching, flocked swabs to replace nasopharyngeal aspirates in respiratory sampling, use of virus molecular subtyping assays, increased efforts to include minorities in research, and better collaboration to encourage research funding.

2 Acknowledgements

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3 Declaration of Work Done by the Author

My contributions to the study included, 1) helping draft the study protocol that was used, along with the subsequent amendments to the protocol, 2) creating the consent forms, case report forms, and advertisement posters, 3) recruiting patients and students, 4) collecting clinical samples, 5) all laboratory work, 6) compiling, analysing, and interpreting data.

Dr. Manish Pareek drafted the bulk of the protocol and subsequent amendments, presented the protocol to the Research Ethics Committee, created information sheets, recruited patients and students, and clinically assessed the health of students who became ill. Dr. Helen M. Dillon drafted the first protocol that received ethical approval, but that was superseded by the one eventually used in the study. She also recruited patients and students, and collected clinical samples during the second year of research. Prof. Karl G. Nicholson helped draft the protocol and provided guidance in creating information sheets and consent and case report forms. Dr. Iain Stephenson conceptualized the project and wrote the first draft of the protocol.

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7 List of Abbreviations

A&E	Accidents & Emergencies
ADE	Antibody-Dependent Enhancement
ARD	Acute Respiratory Disease
AARD	Adenovirus-associated Respiratory Disease
ARDS	Acute Respiratory Distress Syndrome
ARI	Acute Respiratory Infection
BLAST	Basic Local Alignment Search Tool
BMT	Bone Marrow Transplant
CAU	Children's Assessment Unit
CCU	Common Cold Unit
cDNA	Complementary Deoxyribonucleic Acid
CF	Cystic Fibrosis
CHF	Congestive Heart Failure
CNS	Central Nervous System
COPD	Chronic Obstructive Pulmonary Disease
CoV	Coronavirus
CPD	Cardiopulmonary Disease
CPE	Cytopathic Effect
CRF	Case Report Form
Ct	Cycle Threshold
CTL	Cytotoxic T-lymphocyte

DNA	Deoxyribonucleic Acid
E	Envelope Protein
ED	Emergency Department
EKC	Epidemic Keratoconjunctivitis
EV	Enterovirus
F	Fusion Protein
Flu	Influenza
FluA	Influenza A
FluB	Influenza B
FluC	Influenza C
G	Attachment Protein
GCP	Good Clinical Practice
HA	Haemagglutinin
HAdV	Human Adenovirus
HCoV	Human Coronavirus
HCW	Healthcare Worker
HE	Hemagglutinin-Esterase Protein
HI	Haemagglutination Inhibition
HIV	Human Immunodeficiency Virus
hMPV	Human Metapneumovirus
HPA	Health Protection Agency
HSCT	Hematopoietic Stem Cell Transplant

ICAM-1	Intercellular Adhesion Molecule 1
ICU	Intensive Care Unit
IFITM	Interferon-inducible Transmembrane Protein
IFN	Interferon
IFN-α2	Interferon Alpha 2
IFN-λ	Interferon Lambda
IFNAR	Interferon Alpha/ Beta Receptor
IHR	International Health Regulation
ILI	Influenza-like Illness
IQR	Interquartile Range
IRF-7	Interferon Regulatory Factor 7
IV	Intravenous
JAK-STAT	Janus Kinase Signal Transducer and Activator of Transcription
KSA	Kingdom of Saudi Arabia
L	RNA Polymerase Protein
LDL-R	Low-density Lipoprotein Receptor
LOS	Length of Stay
LRI	Leicester Royal Infirmary
LRTI	Lower Respiratory Tract Infection
M	Matrix or Membrane Protein
MAb	Monoclonal Antibody
MERS-CoV	Middle East Respiratory Syndrome – Coronavirus

MFT	Miniature Field Trial
N	Nucleoprotein or Nucleocapsid
NA	Neuraminidase
NAI	Neuraminidase Inhibitor
NEP	Nuclear Export Protein
NI	Nosocomial Infection
NP	Nucleoprotein
NPA	Nasopharyngeal Aspirate
NPW	Nasopharyngeal Wash
NS1	Non-structural Protein 1
NS2	Non-structural Protein 2
OAS	2'-5' Oligoadenylate Synthetase
OR	Odds Ratio
P	Polymerase or Phosphoprotein
PA	Polymerase Acidic Protein
PB1	Polymerase Basic Protein 1
PB2	Polymerase Basic Protein 2
PCF	Pharyngoconjunctival Fever
PCR	Polymerase Chain Reaction
pDCs	Plasmacytoid Dendritic Cells
PFP-2	Purified Fusion Protein 2
PICU	Paediatric Intensive Care Unit

PIV	Parainfluenza Virus
PKR	Protein Kinase Receptor
PPE	Personal Protective Equipment
R	Risk
R_0	Basic Reproduction Number
RH	Relative Humidity
RNA	Ribonucleic Acid
RNAseL	Ribonuclease L
RNP	Ribonucleoprotein
RR	Risk Ratio
RSV	Respiratory Syncytial Virus
RT	Reverse Transcription
RT-PCR	Reverse Transcription Polymerase Chain Reaction
RV	Rhinovirus
S	Spike Protein
SARS	Severe Acute Respiratory Syndrome
SCT	Stem Cell Transplant
SD	Standard Deviation
SH	Small Hydrophobic Protein
SOB	Shortness of Breath
$TCID_{50}$	Tissue Culture Infective Dose, 50%
TLR	Toll-like Receptor

TLR-3	Toll-like Receptor 3
TLR-7	Toll-like Receptor 7
TRIF	TIR-domain-containing adapter-inducing interferon-ß
UoL	University of Leicester
URTI	Upper Respiratory Tract Infection
UV	Ultraviolet
VTM	Virus Transport Medium
WGS	Whole Genome Sequencing
WHO	World Health Organization

8 Introduction

8.1 Aims and Objectives

The overall aim of this study was to augment the body of knowledge on the transmission of the common cold, using the TraVerse method. TraVerse is the first method developed to elucidate natural transmission that 'traverses' all known respiratory viruses associated with the common cold. By this method, symptomatic paediatric inpatients act as respiratory virus sources to healthy young adult hosts, during 30-minute interactions, in a setting that realistically simulates the interchange between patients and medical teams in a functioning clinical facility. The objectives were to 1) elucidate the rates of natural respiratory virus transmission, 2) establish definitive mechanisms by which viruses are spread, 3) recognise the risks of transmission by specific mechanisms in healthcare settings, and 4) ascertain the burden of respiratory viral infections in healthy young adults.

8.2 The Common Cold: the forgotten pandemic

The term 'common cold' refers to an acute syndrome of systemic signs and respiratory symptoms, such as fever and cough, which result from viral infections in humans.[1] Documented anecdotal evidence of the illness and its person-to-person transmissibility goes as far back as the ancient seafaring days, when it was observed that mariners at sea were less frequently symptomatic than when on dry land, where the potential for social contact was greater.[1] Signs and symptoms of a cold are generally benign in nature and most often confined to acute upper respiratory illness, although occasional lower respiratory infections also commonly occur.[2] However, it is the cumulative seasonal effects of the syndrome that makes it the leading cause of morbidity and mortality worldwide,[2,3] which has an estimated annual economic burden of 3 billion U.S. dollars in medications for symptom relief; 110 million primary care visits; and 20 million days each of school and work absences, in the U.S. alone.[1]

It is this burden that precipitated the quest to find a cure, bringing about the establishment of the Common Cold Unit (CCU) in Salisbury, England in 1946.[4,5] In order to find that cure, the unit aimed to definitively delineate the mechanisms by which colds are transmitted. Transmission studies at CCU spanned 43 years, with the most momentous findings being that, a 'filterable agent' causes symptoms; this agent is actually not just one pathogen – influenza, but a whole group of as yet unidentified microorganisms that can infect the same person at the same time, and infections result in relatively similar symptoms that include sneezing, runny nose and sore throat.[4,6-8] Regrettably, the unit was decommissioned in 1989, without having found a cure.[4] However, the identification in the 1950s of rhinovirus as the causative agent in most common cold infections[9] resulted in the development of experimental models on transmission of influenza and rhinovirus, concomitant to efforts at the CCU; these models demonstrated the possibility that virus transmission occurred by several different mechanisms.[10-14] Furthermore, longitudinal epidemiological studies in the 1970s and 1980s established the importance of the family unit in the spread of infection, and the disproportionate incidence of disease among younger children within families.[11,15,16]

In the 1980s and 1990s, modern diagnostic methods such as polymerase chain reaction (PCR) not only confirmed influenza and rhinovirus as causative microorganisms,[17] but also enabled the identification of other viral pathogens relevant to the common cold, which were not recognised when using cell culture.[18,19] PCR further enabled the confirmation of the burden of concomitant infection with multiple viruses in the paediatric population, as established in previous studies.[20,21] Most of what is currently known about the common cold and its transmission were derived from these historical events. The derived general knowledge is discussed below and includes: causative viruses, seasonal peaks, age-related incidences, clinical manifestations and possible mechanisms of transmission.

The viruses commonly associated with the common cold syndrome and their respective proportional rates of global infection are: rhino-enteroviruses (40-50%), influenza viruses (25-30%), coronaviruses (10-15%), adenoviruses (5-10%), respiratory syncytial viruses (5%), metapneumoviruses (5%), and

parainfluenza viruses (5%).[1] Seasonally, these viruses have the ability to cause colds in the UK and other temperate climates year-round, although with decreased incidence in the summer. In tropical climates, cold infections persist all year, but some viruses may have increased incidence in rainy seasons.[1] The average annual incidence of infections in preschool children is five to seven episodes, although 10% to 15% will have at least 12 infections per year.[1,16] Incidence decreases to two to three episodes per year in adults, but tend to be higher in those with regular contact with children.[15] Onset of clinical manifestations occurs within three days after infection,[22] and lasts up to one week. Although, in at least 25% of those infected, symptom duration is approximately two weeks but can be longer when more than one virus is involved and when the person is at medical risk of complications.[1,21] With the seemingly singular requirement that a virus successfully infects the nasal epithelium,[1] it is then easily passed on from one person to another by any of three possible mechanisms: directly, through large droplets emitted during normal talking or through coughs and sneezes and indirectly, by inhaling small droplet aerosols or by self-inoculation of the eyes, nose and mouth after touching fomites.[1,23-27] Despite current knowledge, however, finding a cure for common cold symptoms remains elusive; nonetheless, it continues to be of prodigious importance, particularly because an estimated 500 million cold infections occur each year, at an annual cost of US $40 billion in the U.S. alone.[28] Possible hindrances to progress include: the gaps in knowledge of viruses, host and environment, as well as the modest amount of financial resources available for the pursuit of this knowledge.[2,3,11,29]

Gaps in knowledge of the pathogens of the common cold pose a challenge to finding a cure, primarily because of the number of viruses that cause similar clinical pathology either individually, or in association with one another.[11] For example, myalgia is a typical systemic sign in patients with influenza; however, those with colds due to other viruses are also known to occasionally complain of muscle aches.[29] Furthermore, the development of PCR has led to the discovery of many other viruses that cause the common cold, but there is always the possibility that future diagnostic method developments may reveal more pathogens.[29] By a similar token, insufficient knowledge of different human host responses to these

pathogens is an impediment, particularly where age, socioeconomic status and overall health conditions are concerned.[2,29] For example, children have more cold episodes per year than adults; mothers that work outside the home are less susceptible to disease; and those with genetic conditions may be less able to mount an immune response.[29] Because the immune-challenged and those at opposite ends of the age spectrum are most at risk for severe illness, they have been the focus of most studies on the common cold. [30,31] Meanwhile, little attention is given to studies involving healthy young adults, who are of critical importance to global infrastructure.[32] Furthermore, diversities in the environment pose difficulties in determining the primary mode/s of virus transfer and the conditions by which transmission occurs which have a negative effect on the evaluation of effective measures for intervention.[11,33] For example, influenza transmission in guinea pigs are optimal at a temperature of 5°C and a relative humidity between 20% and 35%, however, any variation in these conditions make transmission of virus less likely.[2,34] Compounding all these obstacles is the inability to distinguish if virus, host and environmental effects in experimental conditions are similar to what occurs naturally in infection,[2] because published data[12,13,23,35] are limited to those that are either experimental in nature, or simulations of natural illness that are not necessarily realistic. Finally, despite the global health and economic impact of acute respiratory infections, and even though cost-effective measures for control and prevention are practicable, commitment to investment in research has yet failed to materialise. Thus, it is by "the forgotten pandemic" that scientists refer to the common cold. [3,36,37]

In 2005, the World Health Organization (WHO) International Health Regulations made it an international health law for each country to have a pandemic preparedness plan, brought about by the emergence of severe acute respiratory syndrome (SARS) in 2003, and the re-emergence, and subsequent spread, of avian influenza A(H5N1) in the same year.[38] To aid countries in developing pandemic plans, the WHO provided published guidelines to be used as a framework.[39] The focus of the framework is the control and prevention of pandemic avian flu, which has naturally renewed interest in flu transmission studies and resulted in the earmarking of more than US $6 billion for pandemic response in the U.S. alone.[40]

Meanwhile, funding for respiratory disease remained between 2.5% and 4.5% of total government research spending in countries such as the UK.[36,40] Recent outbreaks of Middle East respiratory syndrome coronavirus (MERS-CoV)[41,42] and severe and fatal strains of re-emerging human adenovirus (HAdV)[43,44] necessitate that plans take into consideration the possibility that the next pandemic may be caused by a common cold virus other than influenza.[39] In order that a successful plan may be devised, two things are essential: 1) a better understanding of the mechanisms of natural human-to-human transmission of respiratory viruses of pandemic potential and 2) the greater availability of funding for research into these mechanisms.

The First essential was addressed in this study, which evaluated the fitness for purpose of TraVerse in determining natural human transmission of respiratory viruses such as, adenovirus (HAdV), coronavirus (HCoV), enterovirus (EV), influenza (Flu), metapneumovirus (hMPV), parainfluenza virus (PIV), respiratory syncytial virus (RSV) and rhinovirus (RV). Symptomatic children were used as sources, because they are known to shed copious amounts of virus for long periods.[9,45-48] Healthy young adults were selected as hosts, because although they are of critical importance to global infrastructure, they are the ones for whom disease severity is not well studied.[32,49] A large, functioning hospital was used as the controlled setting, because it is the place and the staff who work in it for which infection control measures would need to be operational, particularly during a pandemic. The mechanisms of transmission evaluated were large droplet, hand-to-hand, self-inoculation, fomite and aerosol. Since TraVerse is the first realistic model of natural human-to-human transmission evaluated, any indication of the occurrence of transmission made it fit-for-purpose.

It was not intended for this study to address the second essential for a successful pandemic plan and lobby for more research funding, however, it is nonetheless hoped that it would at least open up the possibility.

This thesis is structured such that a literature review on each of the common cold viruses is presented immediately following this introduction, in order of their proportional rates of infection as mentioned above (RV, EV, Flu, HCoV, HAdV, RSV,

hMPV, PIV). The summary of the literature is followed by a description of the research methods used, which included: TraVerse, quantitative and qualitative real-time, reverse transcriptase PCR (RT-PCR), and genetic sequencing. Results will establish that, as far as the author is aware, this is the first study on the real-life, natural transmission of respiratory viruses, and also the study with the most number of consented participants. The results will also establish that TraVerse is fit-for-purpose, in providing observed rates of each of the mechanisms of transmission, in particular: large droplet, hand-to-hand, self-inoculation and fomite. Comparative epidemiological data on virus infection in children and healthy young adults are provided. A discussion of the results in the context of other published transmission studies follows, which is succeeded by difficulties encountered in the study and, finally, by the conclusions and recommendations of the author.

8.3 Literature Review

8.3.1 Picornaviruses

Picornaviridae is a virus family name derived from the small size (30nm diameter) of its members (pico, Spanish for very small). Single-stranded, positive-sense nucleic acid makes up its genome (RNA). It is made up of 12 genera in which belong pathogens that are of recognised importance in human and animal health, including foot-and-mouth disease virus, hepatitis A virus, human enteroviruses, and human rhinoviruses.[50]

RV and EV are the two most closely related genera in the *Picornaviridae* family. Globally, they are the leading causes of the common cold[51] and are the most prevalent respiratory pathogens in the first year of life.[52,53] In children and adults, these viruses have been implicated in the aetiology of otitis media, bronchiolitis and pneumonia, as well as exacerbations of cystic fibrosis (CF) and asthma.[51,54-59] RV and EV were quantitative RT-PCR targets in the study and are discussed in greater detail below.

8.3.1.1 Rhinoviruses (RV)

8.3.1.1.1 General Information

Rhinoviruses belong to the *Enterovirus* genus, the largest in the *Picornaviridae* family. Taxonomically, they were traditionally characterised according to antisera reactivity, resulting in over 100 different serotypes.[60,61] They were further classified based on receptor specificities for entry into the host cell during infection and on sensitivities to antivirals during virus clearance.[60,61] The introduction of molecular techniques such as PCR and genetic sequencing resulted in the discovery of RV species that previously eluded identification, because they do not propagate in cell culture. This brought about the subsequent, and simpler, reclassification of RV into types A, B, and C, based on their genetic and pathogenic homogeneity.[62] The original 102 serotypes were catalogued by the American Type Culture Collection and subtyped into RV-A (n=76), RV-B (n=25) and EV (n=1). On the other hand, species recently identified through molecular methods are

subtyped RV-C (n=49).[9,63] There is further evidence to suggest that there may be an emerging subtype D.[62] Figure 1 is a phylogenetic tree that shows the inferred evolutionary relationships of currently identified RV genotypes.

Genetic recombination among RV types commonly occurs, particularly between RV-A and RV-C, resulting in the evolution of quasispecies.[63-65] Recombination generally occurs due to the misincorporation of RNA during transcription by error-prone RNA polymerase, which has an error frequency of at least one misincorporation per 10^4 nucleotides in RV.[50] Recombination also occurs as a result of frequent infection of hosts by multiple RV types.[50,62,66] RV evolves due to recombination events throughout the whole genome, rather than just in immunogenic regions,[62,66] as commonly occurs with other RNA viruses, although genetic mutations occur more frequently in the 5'-UTR and along its adjacent regions.[63-65]

8.3.1.1.2 Epidemiology

Globally, RV is the prevalent cause of upper respiratory tract infection (URTI) in humans throughout the year.[9] In the UK and other temperate regions, a seasonal pattern in virus isolation is observed in the autumn, winter and spring, with peak activity coinciding with school openings in autumn and three weeks after children return from break in spring.[60] Infections are most pronounced in infants and young children,[63] however, the entire population is susceptible, causing approximately 35-60% of common colds during the entire year and increasing to more than 80% during the spring and autumn peaks.[60,63]

Respiratory symptoms due to RV infection are generally mild and self-limiting in immunocompetent hosts, starting between two hours and two days after infection and lasting up to 14 days.[9] Peak virus shedding occurs at approximately the same time, lasting up to 10 days in the overall population.[60] Generalised symptoms include rhinorrhoea, nasal congestion, pharyngitis, cough, headache, low-grade fevers and malaise.[9] Protracted shedding of a single RV subtype in healthy individuals is uncommon, but not in the immunosuppressed, who are also more severely affected.[63,67,68] Virus clearance follows within three weeks, however, re-

infection with other subtypes can occur, resulting in prolonged illnesses.[60,63,69] When accompanied by acute asthma exacerbations, recovery can take as long as six weeks.[63] Chronic infection is also possible, particularly in lung transplant patients.[60] Frequent recurrence of infection causes strains in global economies, due to time lost at work and school, and increased numbers of medical consultations.[9,60] Episodes of infection average from two to five annually in adults, and approximately 12 per year in children. It is estimated that one to two years of an average person's life is spent in misery due to a common cold, costing the economy approximately US $40 billion annually in the U.S. alone.[9,60]

RV is also associated with infections of the lower respiratory tract (LRTI). In children and adults with underlying health conditions and immunosuppression, RVs are implicated in the aetiology of more serious diseases such as otitis media, bronchiolitis, and pneumonia; and exacerbations of asthma, cystic fibrosis and chronic obstructive pulmonary disease (COPD).[9,60] For those hospitalised with RV infection, children less than five years of age present with bronchiolitis and pneumonia, and approximately 14% are admitted to paediatric intensive care units (PICU).[9] Older children and young adults, on the other hand, are generally

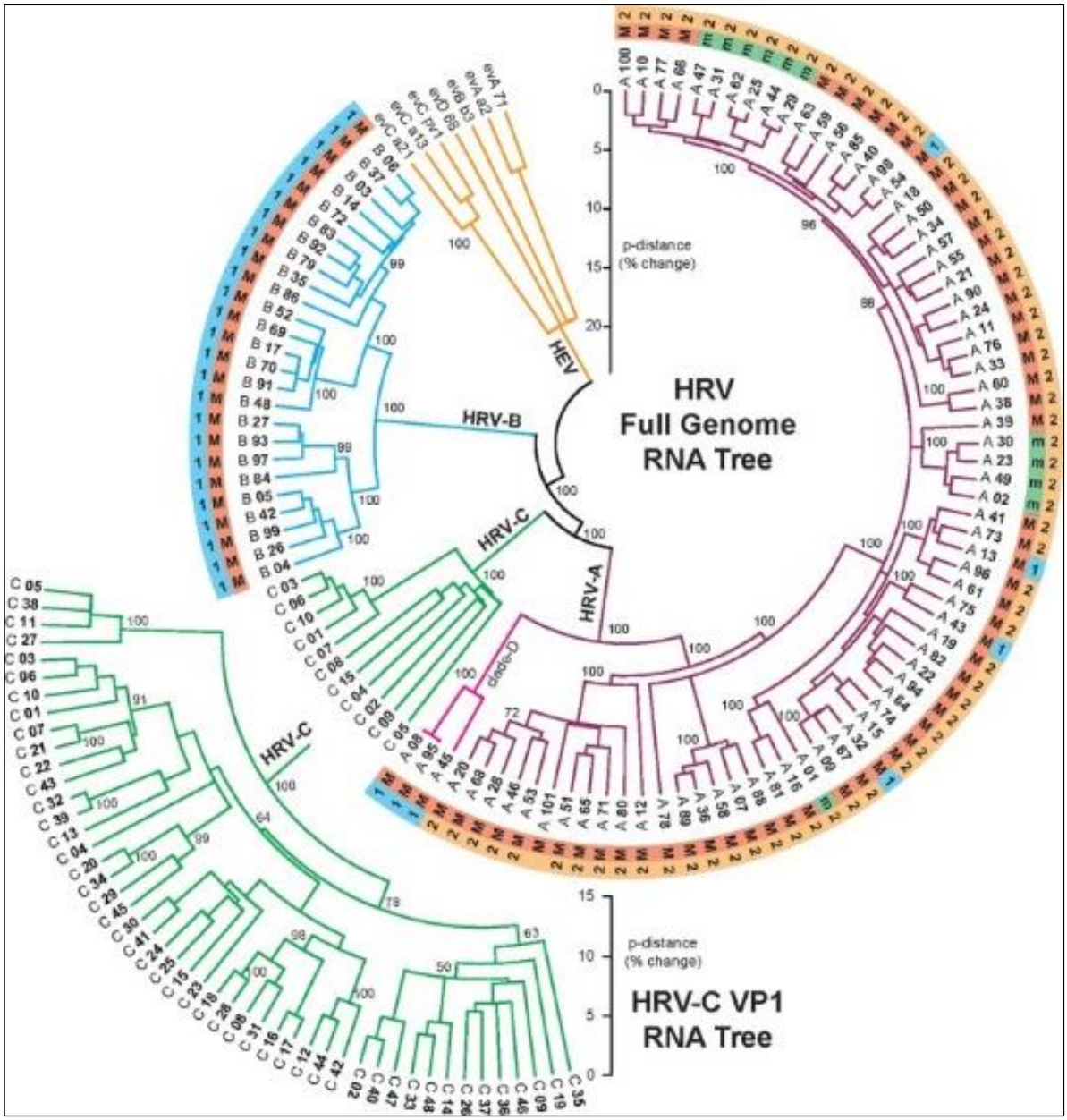

Figure 1 RV Phylogenetic tree [a]

[a] Shown are circle phylogram relationships for known genotypes of RV-A, RV-B, and RV-C. The tree was calculated with neighbor-joining methods from aligned, full-genome RNA sequences and rooted with data for human enterovirus species A, B, and C. The outer ring ("1" or "2") indicates anticapsid drug group types, if known. The inner ring shows members of the major ("M") (ICAM-1) and minor ("m") (LDLR) receptor groups. The RV-C receptor is unknown. Since few RV-C strains are fully sequenced, the determination of relationships among these genotypes relies on partial VP1 RNA data (bottom left). Bootstrap values (percentages of 2,000 replicates) are indicated at key nodes. (Taken from Gern & Palmenberg)[63]

admitted for asthma exacerbations.[9] RV-related asthmatic episodes do not predispose patients to more frequent and severe URTI or to longer lengths of hospital stay than non-asthmatics, however, they are more likely to frequently develop severe LRTI.[9] Elderly patients for whom RV infection induces COPD exacerbations, hospitalisations due to pneumonia and congestive heart failure are common.[9,60,63] Those in closed settings are particularly vulnerable to serious illnesses that could lead to death.[9,60,63] In infants, morbidities include otitis media and severe acute bronchiolitis due to infection with either RV alone or, as is common, along with RSV.[9,60] Bronchiolitis at this early age predisposes one to recurrent wheezing in the first year of life, which may progress to asthma in the early childhood years.[9,60] Infants with underlying pulmonary abnormalities and children with primary immune deficient disorders may develop life-threatening complications requiring PICU admission and mechanical interventions.[60] In CF patients, symptoms of RV illness can be exacerbated by secondary bacterial infections, such as those due to *Pseudomonas* species, leading to disease progression.[60] RV-induced CF exacerbations are not associated with decreased pulmonary function; however, virus pathology in LRTI in this population may be related to species subtype.[9]

Severity of disease has been associated with specific RV subtypes and with viral load.[9,62,70] RV-A and RV-C are indicated in more serious illnesses than RV-B,[71] although severe infections with RV-B generally require mechanical ventilation.[71] RV-C is implicated in approximately 50% of paediatric RV infections, and often linked with wheezing episodes and exacerbations of asthma and CF.[70,71] RV-C is also isolated in clinical specimens such as bronchoalveolar lavage, pericardial fluid, blood, urine and stool, suggesting that it may cause systemic debilities.[9] Recent studies indicate that RV-A may give rise to similar clinical manifestations as RV-C.[63] Disease severity can also be predicted by the amount of infectious virus particles present in nasopharyngeal exudates.[9,72] Children and adults with a viral load of at least 10^7 copies/mL are usually hospitalised with acute respiratory infection (ARI).[9,73] In children older than 11 months with LRTI, viral load is likewise predictive of illness severity scores.[9,74] Meanwhile, lower viral loads between 10^3 and 10^4 copies/mL have been correlated with symptom

development,[9,75] but consecutive sampling has not successfully determined when illness will resolve.[9,72] Caution in correlating viral load to illness severity is needed, however, because definitive interpretation is made difficult by differences in sample collection and laboratory methods.[9]

The wide spectrum of illnesses caused by RV suggests that host and environmental factors may be equally relevant in disease presentation.[9,63] Host risk factors for wheezing following RV infection include maternal atopy, young age, history of hypersensitivity reactions, previous wheezing and asthma.[63] Environmental factors include tobacco smoke, air pollution, and stress.[9] Large sets of data from longitudinal studies are needed to validate these findings.[9,63]

8.3.1.1.3 Infection and Immunity

Humans are the primary RV hosts. Entry and replication in the host principally occurs in the nose and eyes, through either the intercellular adhesion molecule 1 (ICAM-1) or the low-density lipoprotein receptor (LDL-R).[60,63] RV carriage occurs at doses considerably less than one tissue culture infective dose ($TCID_{50}$). Oral entry through inhalation is also possible, but doses up to 10,000 more than those through the eyes and nose are required to cause disease.[60,63]

Host infection primarily occurs in the upper airways.[63] Air-liquid interface culture methods show that RV thrives in the undifferentiated epithelial monolayer and occasionally in the sub-epithelial layer of the airways.[76] On the other hand, viral replication in differentiated cells occurs only when there is damage to the epithelium, suggesting that resistance to RV is a function of barrier protection by the epithelial layer.[63,76] In the lower airways, RV replicates best in the large- and medium-sized airways, where temperatures are between 33˚C and 35˚C.[77,78] Cell culture methods show that RV replicates as well, if not better, in lower airway epithelial cells than in upper epithelial cells, which led to the understanding that RV infection is not limited to the upper respiratory tract.[63,69,77-79]

Asymptomatic RV infection regularly occurs, particularly in infants, where the rates range from 25% to 44%.[63,80] The high rates could be explained by both the age-related susceptibility to RV, which is generally lower in the first six months

after birth but tend to increase considerably throughout infancy and early childhood, [60,63] and the fact that mild symptoms may not be easily identified in this group.[63] Asymptomatic infection is said to have occurred when virus is shed during either the prodromal or recovery periods of a respiratory illness.[80,81] In healthy adults and the elderly, limited studies on asymptomatic RV infection suggest a lower prevalence of up to 2%, but may be higher in household settings where children reside.[9,82-84] There is no evidence of virus carriers with persistent, low-level shedding and viral load is not a good indicator of the development of a symptomatic or asymptomatic phenotype.[84] However, both phenotypes are able to elicit an immune response.[84,85]

Immune responses to RV are regarded as the underlying causes of the multitude of symptoms associated with infection.[9,63] It is seldom observed that RV carriage results in injury to the respiratory tract or in the increased numbers of infected epithelial cells. Tissue oedema and neutrophil and mononuclear cell levels of no notable importance typify pathology during peak infection.[9,63] On the other hand, host innate pro-inflammatory response involving interleukins, kinins, leukotrienes and histamines cause vasodilation and oedema, which correlate to disease severity and viral load.[9,63] Epithelial cells undamaged by allergens and pollutants may prevent RV infection but activate a variety of signalling pathways resulting in pro-inflammatory response, antiviral activity and cell death, which also negatively influence disease pathology.[9,63]

Cellular immune response involves macrophages, neutrophils and lymphocytes.[63] Macrophages are much like epithelial cells in producing cytokines, chemokines and antivirals during RV infection, albeit through a different signalling pathway.[86-88] Synergistic epithelial and macrophage response results in the secretion of types I and III interferons (IFN), which are critical in early innate immunity to RV.[63,89] Neutrophils are the most abundant cells in RV nasal secretions.[63,90,91] Production of neutrophil precursors in the bone marrow is stimulated within 24 to 48 hours of infection after which, neutrophils are recruited to the upper- and lower-respiratory tract.[63] Neutrophilic response accompanies pro-inflammatory and antiviral activities that correlate with acute cold symptoms.[63,90] Lymphocytes (T-cells) decrease in numbers in the blood and increase in nasal secretions for a short

period during an RV-induced cold.[63] They are critical in virus clearance, and T-cell immune deficiencies can lead to protracted illnesses and severe clinical manifestations.[63,67] T-cell response can be serotype-directed and an aggressive initial antiviral activity is associated with milder symptom development.[63]

Humoral immune response, involving the secretion of both neutralizing serum IgG and secretory IgA antibodies in the airways, occurs within 7 to 14 days following infection.[9,63] Antibody titres persist for at least one year after induction, providing serotype-specific protection from re-infection and decreased disease severity while levels remain elevated.[9,63,92,93] There is very little evidence of cross-neutralizing antibody activity to RV, which makes vaccine development tedious, given the 102 identified serotypes with no common antigen.[9,94]

8.3.1.1.4 Transmission

Current literature on RV transmission is elucidated from household studies and research involving artificial virus infection of healthy adult hosts, [13,23,35,84,95,96] such as research on families by Peltola, et al.,[84] and the aerosol study by Dick, et al.[23] Hence, there is a gap in knowledge of the natural human-to-human transmission of RV in symptomatic cases that result in medical consultations and hospitalisations, and the infection rate in the healthy young adults involved in the care of these patients. Furthermore, previous RV transmission studies occurred before molecular laboratory methods for identifying viruses became widely available.[13,23,35,84,95,96] Examples include Gwaltney, et al.'s experiments with environmental surfaces in 1982 [35] and Hendley et al.'s research on self-inoculation with RV in 1973.[96] Therefore, data from these are limited to RV-A and RV-B that propagated in cell culture. Inferences from household and experimental studies are delineated in the ensuing paragraphs.

RV transmission is presupposed to be essentially inefficient, because it is highly dependent on several personal and social factors such as, young age of source and host, source symptom array and high titre shedding, host seronegativity, personal hygiene and crowding.[13,63] Studies suggest that children transmit disease to other children and to adults readily, although adults do not pass on infection to children

and other adults as easily.[63,84] In school and childcare facilities, transmission is largely influenced by social factors and children's hygiene.[60] In households, infection of adults by children takes place between five and ten days after the index case becomes ill, depending on viral load and duration of shedding by the child.[60] Healthcare-associated RV transmission has been known to occur in paediatric wards and elderly housing facilities and is suggested to be attributable to social factors.[9] RV epidemics are unusual, unless associated with influenza-like illness (ILI), commonly observed in elderly housing.[63,97]

Transmission through exposure to large droplets has been observed,[63] although, the primary mechanism is suggested to be through either fomites or aerosols.[9,35,60,96,98] Nonetheless, duration of contact is established to be the best predictor of RV transmission.[13] RV is detected on hands at an estimated rate of 40%, and in up to 6% of household objects.[9] At room temperature, RVs can remain active on untouched skin for two hours and on surfaces for several hours, however, transfer onto hands, nose, and eyes could occur within just a few seconds.[60,63,96] Aerosol transmission has been observed to occur at a rate of approximately 56% among adults with close contact for prolonged periods.[9,23] A transmission rate of <10% was observed among adult interactions that lasted between 15 minutes and 3 hours;[99] the rate was 30% for close personal contact of long duration such as common in couples,[100] and up to 100% for adults in daily contact in crowded environments.[101]

8.3.1.1.5 Prevention and Control

There is no vaccine for RV. Barriers to vaccine production include the large number of serotypes with no common antigen, deficient epidemiological data on the most actively infecting strains and the lack of appropriate animal models.[9,102] Several attempts were made in developing antivirals but also without much success.[9,63] Antiviral agents include some that act on the virus by interfering with its lifecycle and others that function by mimicking natural host antiviral production. RV lifecycle inhibitors include capsid-binders such as Anti-ICAM-1 that prevent virus from binding to its cellular receptor. [9,103] However, these are impeded by the development of resistance by RV.[9,103] Mimic host immune inducers

include alpha-2 interferons (IFN-α2) and first-generation antihistamines that prevent, shorten the duration, and limit the severity of infection. [104-109] However, they can be toxic and cause side effects that include nosebleeds and nasal irritation.[9,63,104-109]

In the absence of an effective vaccine and antiviral therapy for RV, homeopathic medicines in the form of topical zinc and nutritional supplements that include vitamin C are used to prevent virus infection and alleviate common cold symptoms.[63] However, zinc is associated with the development of anosmia[63] and the medical benefits of vitamin C relative to RV infection is determined to be unsubstantiated.[9,63] On the other hand, decongestants, topical ipratropium and aromatic petroleum jelly rubs may provide relief for individual cold symptoms, including nasal stuffiness and rhinorrhoea.[63,110,111] The heavy metal zinc is likewise associated with relief of cold severity in some and shortened duration of illness in adults.[112-114] However, caution is warranted in the use of lozenges with high zinc content as they may have harmful side effects.[115]

For the prevention and control of RV transmission, public health measures to alter social behaviour are in place, including distancing, wearing of respiratory masks and hand washing.[9] Social distancing measures such as cancellations of school and large public gatherings have been shown in mathematical models to decrease secondary attack rates and epidemic spread of different respiratory viruses by up to 90%.[9,116,117] However, real-life scenarios are proving much more difficult to assess.[118,119] Respiratory masks are effective personal protective equipment (PPE) against respiratory viruses, particularly in healthcare settings. Although, it is questionable whether the choice of one designed to filter up to 95% of very small particles (N95 respirator) over a standard surgical mask has added benefits in occupational settings.[119,120] Finally, hand washing with soap and water or alcohol-based products are shown to effectively reduce RV transmission by self-inoculation and fomite contamination. However, these findings are in young children, who may be the greatest virus shedders but are also the least able to wash their own hands.[9,119,121,122]

8.3.1.2 Enteroviruses (EV)

The *Picornaviridae* family shares similar characteristics among its 12 genera, hence, the description of rhinoviruses in section 8.3.1.1 also applies to enteroviruses.

8.3.1.2.1 General Information

The first enteroviruses to be discovered were coxsakievirus and echovirus, around the time of the poliomyelitis outbreak in the U.S. in 1947.[48] More recently, several other strains of enterovirus were identified using PCR and DNA sequencing methods, leading to their taxonomic classification into species EV-A, EV-B, EV-C and EV-D.[48] Sequencing has further accentuated the pronounced genetic similarities between EV and RV, resulting in their re-classification into the same genus - *Enterovirus.*[48]

8.3.1.2.2 Infection and Immunity

The primary mode of host entry is via the digestive tract, through oral intake.[123] Virus replication occurs in cells along the digestive tract, concurrent to replication along the respiratory tract.[123] Some strains of EV are, therefore, also known to cause respiratory infections characterised by generally mild upper respiratory symptoms during the summer and autumn seasons of the northern hemisphere.[48,60,123] Severe respiratory infections in children can develop into pneumonia and bronchiolitis, while infected infants may not develop any respiratory symptoms at all but continue to shed viruses through stool and nasopharyngeal exudates.[123]

8.3.1.2.3 Transmission

Available literature on EV transmission is elucidated from longitudinal household studies.[95,124] Therefore, this is the first study to explore natural respiratory EV transmission in a non-household setting.

EV transmission occurs primarily by the faecal-oral route.[48,125] Respiratory transmission occurs mostly in regions with more developed sanitation, through similar mechanisms used by RVs.[48,125,126]

Children generally spread disease in the household.[48,95,127] Household transmission of various species is greatest among non-immune mothers in poorer families and with the largest number of children between the ages of 5 years and 9 years.[48,124] Healthcare workers (HCW) may also propagate disease spread in neonatal wards, where nosocomial outbreaks are also prevalent.[48] There are other EV species that are more often detected in bone marrow wards and that may be fatal.[48,128,129]

8.3.1.2.4 Prevention and Control

There are currently no available therapies for EV infections.[48] Although, there are efforts towards vaccine prevention of the often-fatal EV71 strain, which are proving effective in animal studies.[130-132] Current in vitro and animal model studies on antiviral drugs are also showing promise.[48]

8.3.2 Orthomyxoviruses

The family *Orthomyxoviridae* is characterised by viruses whose ribonucleic acid (RNA) genomes are negative-sense, single-stranded and segmented.[133] Six genera belong to the family and include, *Influenza A* (FluA), *Influenza B* (FluB), *Influenza C* (Flu C), *Thogotovirus*, *Isavirus* and *Quaranfilvirus*.[133] *Influenza* viruses cause disease in vertebrates including birds, humans, pigs and other mammals;[134] *Thogotoviruses* are transmitted by arthropod vectors to vertebrates and invertebrates;[135,136] *Isavirus* infects salmon;[137] and the newly included *Quaranfilvirus* primarily infects birds and arthropods, but has also been demonstrated to infect humans.[138] *Influenza* viruses are documented to have caused diseases for at least 400 years and so are the most studied in Orthomyxoviridae.[139] They are also target organisms in this study, and therefore, subsequent sections discuss the three flu genera.

8.3.2.1 Influenza Viruses (Flu)

8.3.2.1.1 General Information

Human influenza viruses are responsible for increased morbidities and mortalities worldwide, due to their ability to cause annual epidemics and occasional pandemics.[140] They are a threat to global health in their ability to cross species barriers and directly infect animals, particularly avian and porcine species, and humans at the same time. By extension, flu viruses are a serious risk to global economy in terms of medical consultations, treatment, and loss of productivity due to absences at work.[140]

Influenza is a vaccine-preventable disease, owing mostly to the large body of knowledge about the virus evolutionary processes.[140] Flu viruses evolve by accumulating mutations overtime, at an estimated rate of 5×10^{-4} to 8×10^{-3} nucleotide substitutions per site per year (drift strains),[141-143] and by the reassortment of viral genes between 2 or more influenza species (shift organisms).[140] A schematic of the creation of a novel influenza A subtype through antigenic drift and shift is shown in figure 2 below.[144] At the amino acid level, strains that primarily infect aquatic avian species evolve slowly, suggesting that virus is well adapted to these hosts.[140] In terrestrial birds, however, selective pressure may lead to amino acid changes that create highly pathogenic and rapidly emerging flu strains that cause outbreaks.[140]

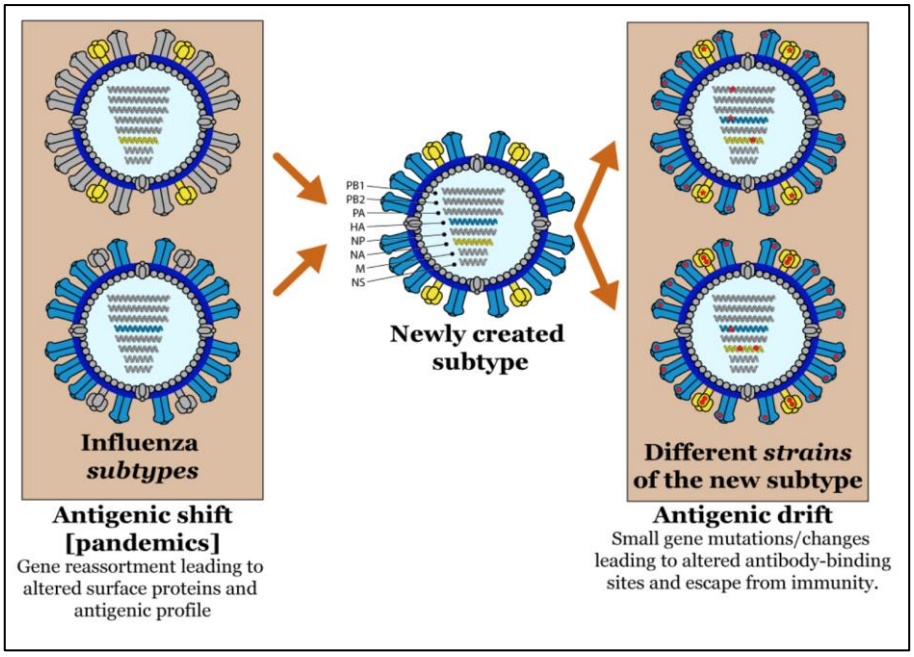

Figure 2 Schematic of Influenza A Antigenic Shift and Drift[b]

Common to FluA, FluB and FluC are an enveloped structure derived from the host, surface envelope glycoproteins, and the RNA genome, but each genus can be distinguished according to their antigenicities, genome, structure, host species, epidemiology and clinical manifestations.[139] FluA and FluB are of most clinical importance, because they cause severe respiratory syndromes in humans.[145] FluA viruses are further classified into subtypes, according to the antigenic properties of their surface hemagglutinin (HA) and neuraminidase (NA) genes. There are currently 17 HA subtypes (H1 to H17) and 10 NA subtypes (N1 to N10).[133,143,146] FluB, on the other hand, remained homogeneous until they diverged into the antigenically different Yamagata and Victoria lineages in the 1970s, hence, FluB is subtyped as either Victoria or Yamagata.[133,145] There are currently no known antigenic subtypes for FluC.[140]

Structurally, flu viruses are complex.[133] There is an envelope lipid bilayer that is derived from the host cell as progeny virions are released from the cell nucleus. On the surface of this envelope are found the glycoprotein receptors HA and NA and the transmembrane proton channel matrix (M2) protein.[133,147] Just beneath the

[b] Taken from MacKay, 2013[144]

envelope is another matrix protein (M1), which binds both the envelope and the RNA core.[148] The virus core is a complex of ribonucleoproteins (RNP) that include the RNA segments, 2 polymerase basic proteins (PB1 and PB2), a polymerase acid (PA), a nucleoprotein (NP) and non-structural and nuclear export protein (NS1 and NS2/ NEP).[133,149] A schematic of FluA structure in figure 3 shows its two surface glycoproteins, the matrix protein and ion channel, and the eight internal genes.[144]

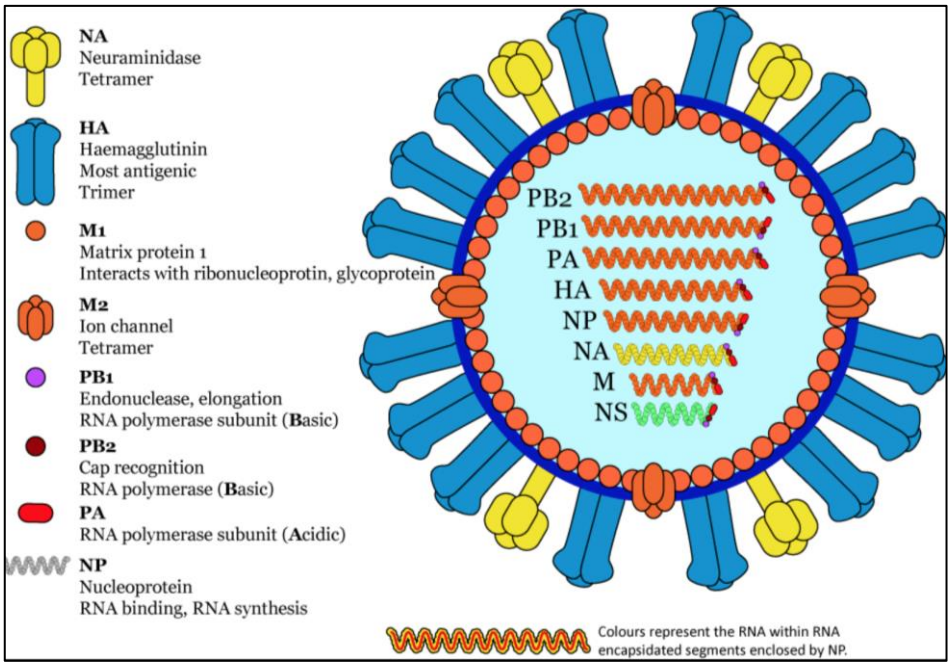

Figure 3 Influenza A Structure Schematic[c]

8.3.2.2 Epidemiology

Epidemiologically, human flu viruses are also complex.[140] Their distribution patterns vary in different climates and geographical regions, they evolve through antigenic shift and drift to elude host immune systems, and they also cause a wide range of diseases that contribute to increased worldwide morbidities and mortalities every year.[140]

[c] Taken from Mackay, 2013[144]

Globally, flu infections are identified throughout the year. However, there are distinct epidemic patterns between temperate and tropical regions.[150] Influenza epidemics generally occur seasonally during winter in temperate regions where they may last from December to May and peak between January and April in the northern hemisphere,[151-154] while in the southern hemisphere, the season lasts from May to September.[155,156] In tropical and subtropical regions, on the other hand, influenza activity is most noticeable during the rainy seasons.[157-159] Epidemics also tend to occur irregularly, albeit at less noticeable rates than in temperate areas, due to the yearlong flu prevalence in these places.[150,159-161]

Prior to the 2009 pandemic, the epidemic strains that co-circulated every season were FluA(H3N2), FluA(H1N1) and FluB. Since 2009, however, epidemic H1N1 has been replaced by the pandemic strain A(H1N1)pdm09 in seasonal circulation patterns.[140] Studies on flu global distributions suggest that seasonal H3N2 and H1N1 may have originated from the tropical regions of Southeast Asia and spread towards areas of temperate climate.[150,152,162-165] The tropics generally experience yearlong circulation of multiple flu strains,[166] while temperate regions such as North America and Europe find that the three seasonal strains circulate during the earlier part of an epidemic season, but the dominant variant is the one sustained throughout the height of the epidemic.[167]

8.3.2.2.1 Antigenic Drift and Shift

Antigenic drift is the gradual accumulation of point mutations in FluA and FluB HA or NA genes.[168] FluA drift strains are often a result of positive selection of mutations in the immunogenic (HA1) portion of the HA gene that, although they spontaneously arise, they nonetheless circumvent the lasting immunity that results from infection.[169] In humans, HA or NA drift mutations occur less than 1% per year, however, this rate is enough to bring about outbreaks for the duration of up to 5 epidemic seasons, until it is replaced by a new variant.[140] In contrast, antigenic shift is a major change in the HA or NA gene that results in a variant that is antigenically different from the circulating strain.[140] These mutations tend to cause pandemics because they typically result in the creation of a new subtype, to which everyone is immunologically naive.[140] There were 4 pandemic shifts in the

20th century, all of which were sudden occurrences at unpredictable intervals: The H1N1 Spanish flu of 1918 was followed by the Asian H2N2 in 1957, which was then followed by the Hong Kong H3N2 in 1968, and then by the Russian H1N1 in 1977.[170] The first pandemic of the 21st century occurred in 2009, with another H1N1 strain (pdm09). This was an exception in that it replaced a strain of the same subtype that has been circulating since 1977, which contradicts the shift strain definition.[140] Recent studies suggest that this may either be because A(H1N1)pdm09 derived from the reassortment of genes from swine, avian and human strains, rather than the more common event between human and avian genes,[171-174] or because A/1977(H1N1) was not an evolved species, but rather the result of either a laboratory accident or a vaccine trial or development that has gone awry.[175] It is now acknowledged, therefore, that flu pandemics may occur not just through antigenic shifts that result in a different subtype, but also by any means where antigenicity differs sufficiently to be able to evade the immune response of most human hosts.[140]

8.3.2.2.2 Morbidity and Mortality in Humans

The annual global incidence of flu is approximated to be 20% to 30% in children and 5% to 10% in adults.[166] Attack rates are highest in school-aged children and their symptoms are also generally more severe than in healthy young adults.[176-178] School-aged children are also more likely to spread the disease, thus, increased school absences in winter are used as indicators for the beginning of the flu epidemic season.[140,179] An estimated 3 to 5million of very young, very old and chronically ill people are at high-risk for severe illnesses that require hospitalisation and up to 500,000 of these cases result in death.[166,180-183] Disease severities differ for each epidemic season and are generally dependent on host age at infection, pre-existing immunity to the circulating strains and virus strain virulence.[176,184-186] Elderly people with cardiopulmonary disease (CPD) are most at-risk of pneumonia,[187] while pregnant women and individuals with malignancies and metabolic diseases are at-risk of increased infections.[188-190] In industrialised nations, such as the U.S., the highest rates of flu hospitalisations are in children younger than 2 years old, while approximately 90% of deaths are observed in those aged ≥65 years. [179,191-194] Deaths due to flu epidemics have increased in the

past decades and is currently estimated to be between 20,000 and 40,000 annually, primarily due to an ageing society and the increased numbers of immunocompromised individuals.[191,194]

Flu pandemic events follow the epidemic curve in that shift strain emergence in a community is followed by an acute peak in infection and then by increased rates in mortalities.[140] Exceptions to this are the pandemics of 1918 and 1957, which had second peaks that caused more severe disease than the first.[195-197] The Spanish flu pandemic of 1918 was the most fatal, resulting in 20 to 50 million deaths worldwide.[198] In comparison, U.S. mortality rates in subsequent pandemics were: 70,000 in 1957, 33,800 in 1968 and 12,470 in 2009.[140,182] Note that, although excess deaths during pandemics are greater than in epidemics, the combined deaths in inter-pandemic years still far exceed those sustained during pandemics.[140,182] Age-related excess mortalities during pandemics differ from epidemics in that, healthy older children and young adults are also severely affected, for reasons that have not yet been determined, but that further compounds the unpredictability of the virus.[140,183,199] In 1918, mortalities were typically high in the elderly; however, deaths in healthy young adults were noticeably higher.[140] Likewise, in 1968, approximately 50% of deaths were in groups younger than 65 years old.[182] Finally, in 2009, hospitalisations and mortalities were disproportionately highest in children and young adults.[199,200]

The global economic burden related to consultation fees, hospitalisations, diagnostic testing and clinical treatment were estimated in 1986 to be upwards of 1 billion U.S. dollars annually, while indirect costs related to loss of income due to absenteeism, decreased productivity and loss of life was approximately 2 to 4 billion U.S. dollars for each pandemic season.[201] Twenty years later, these estimates have increased to upwards of $10 billion for direct costs and $16 billion for indirect expenditure annually.[202] In low- to middle-income nations, it is presumed that most deaths due to influenza occur in children.[166] However, the exact nature of epidemic flu burden in these countries is not known, hence, it is acknowledged that flu contributions to global morbidity, mortality and economic burdens are underappreciated and this shortcoming must, therefore, be addressed.[166,203]

8.3.2.3 Infection and Immunity

Waterfowl are considered to be the natural reservoirs for FluA viruses. All characterised HA and NA subtypes are sustained in these species, without causing disease in the population.[143,204-208] However, FluA viruses are also capable of infecting humans and other wild and domestic animals, in which they cause disease. [209-213] Human FluA pandemics are generally associated with H1 and H3 subtypes. However, avian H5, H7 and H9 are of particular concern, because they have also directly infected humans although they have not, thus far, sustained the capacity for human-to-human transmission.[209-217] In comparison, FluB primarily infects humans, but has occasionally been isolated in certain species of seals.[218,219] FluC, on the other hand, infects humans as well as porcine and canine species.[220-222]

The seasonally circulating strains FluA(H3N2), FluA(H1N1) and FluB cause similar symptoms, although severe disease and death are more common with H3N2 infections.[223-226] A closely related drift strain can cause reinfection with the same subtype, although, clinical illness is generally less severe than during primary infection.[223,224,226,227] Signs of FluA infection in humans can range from asymptomatic carriage, to fatality due to complications resulting in pneumonia.[140] The typical clinical manifestation in uncomplicated cases is tracheobronchitis, accompanied by partial involvement of the lower respiratory tract.[140] Disease often begins abruptly after an incubation period of one to five days,[228] and is characterised by systemic signs that include fever that peaks between 38˚C and 40˚C within 24 hours, frank chills with shaking, arthralgia and myalgia that may involve the extremities or the back, headache, malaise and anorexia.[139,140] Systemic symptoms often last as long as the fever persists; generally within 72 hours, but no longer than 8 days.[139,140] Respiratory symptoms that include dry cough, severe pain in the back of the throat, sneezing and coryza are also observed at the onset of disease, but are outweighed by the dominant systemic signs that distinguish flu infections from other viral respiratory diseases.[139] Symptoms could last as long as two weeks and may be accompanied by lethargy.[140]

Severe flu disease resulting in complications and death are at highest risk for children <2 years old, the elderly, pregnant women and people with chronic conditions, metabolic diseases or weakened immune systems.[166] Complications may be pulmonary or extrapulmonary in nature. Those that involve the lungs include primary influenza viral pneumonia, secondary bacterial pneumonia, croup in children and acute exacerbations of chronic bronchitis, asthma or CF.[229-234] Non-pulmonary health difficulties include viremia, inflammation and degeneration of lower leg muscles,[235,236] Reye's syndrome in FluB infections in children aged up to 8 years that reside in rural areas, and in children treated with aspirin to reduce fevers,[237,238] and complications involving the CNS, including Guillain-Barre' syndrome, transverse myelitis and encephalitis.[239-241] In pregnant women, the risk of developing fatal influenza disease is highest between the second and third trimesters,[190] however, risk of congenital defects in the foetus has not been consistently established.[242-245]

FluB infections primarily occur in older children and adolescents.[246,247] They cause similar clinical manifestations in humans as that of FluA. However, they very rarely cause epidemics and the total burden in the paediatric population is estimated to be only one-third that of FluA.[248] Severe disease is also likely, although at rates that are approximately fourfold less than that in FluA infections.[246,249-251] On the other hand, FluC is only known to cause sporadic URTI, which rarely develop into serious LRTI.[252,253] Most children infected with FluC are aged younger than 6 years[254] and an estimated 96% of young adults have FluC antibodies.[253]

A substantial amount of what is known about host immune response to flu infection is based on mouse models.[140] Although, this is currently being augmented by the use of mathematical models, to establish a more complete description of host immune pathways following either natural infection or flu vaccination.[255-261] It is reasoned that the short period between infection and onset of symptoms in mice implies the importance of either innate immunity or a similar intrinsic system of recognising flu particles.[140] Innate immune responses to flu include pathogen recognition that induces the upregulation of toll-like receptor 3 (TLR3) in airway epithelial cells[262-265] and TLR7 in plamacytoid dendritic cells (pDCs).[264-

[268] TLR3 and TLR7 stimulate the expression of type 1 IFN and pro-inflammatory cytokines and chemokines, through the activation of transcription factors, following RNA recognition. TLR3 recognises double-stranded RNAs and engages the adaptor molecule TRIF to activate transcription factors IRF3, NFκB and activator protein 1.[262,269,270] On the other hand, TLR7 recognises single-stranded RNAs and activates IRF7 and NFκB with the help of the adaptor protein MyD88.[266,271] TLR7 is abundantly expressed in pDCs and is, therefore, most often associated with hyper-secretion of IFN-α following flu infections,[266,271] while TLR3 is believed to be most responsible for the induction of pro-inflammatory NFκB and the subsequent immunopathology in some flu cases.[263,272]

Type 1 IFNs have autocrine and paracrine functions that are activated upon binding to the ubiquitous IFN-α/β receptors (IFNAR).[273] Studies suggest that IFNAR deficiencies predispose increased susceptibilities to flu infections,[273,274] but may also increase survival rates from flu complications that lead to secondary bacterial infections.[275] IFNAR activation upregulates the JAK-STAT pathway, which induces the expression of IFN-stimulated genes, by forming a transcription factor complex.[276,277] IFN-stimulated genes include those that encode TLRs, PKR, OAS, RnaseL, ISG15, Mx, Viperin and IFITM antiviral proteins.[140]

A recent study in mice found that type III lambda interferon (IFN-λ) also has a role in innate immunity to flu viruses.[278,279] IFN-λ is similar in function to IFN-α, activating similar signalling pathways. It is suggested to be superior to IFN-α in that, it promotes antiviral activity in epithelial cells without inducing a pro-inflammatory response that could then lead to cell damage or cell death. It is further suggested that IFN-λ is the first line of antivirals against flu, and that only when it fails to block disease progression that type 1 IFN-α is activated.[278,279] Therefore, IFN-λ has implications for the therapeutic prevention and control of flu infections.[278]

Host adaptive immunity to flu infections involves both antibody and T cell-mediated responses to eliminate virus particles.[280] Animal studies suggest that disease is a consequence of the absence of both B and antigen-specific T effector cells,[280-282] while the presence of either cell may be able to control infection, but

not necessarily clear the virus.[283-287] Adaptive immunity to flu is strain-specific and may be long-lasting. [288] However, there is evidence of cross-reactive immunity among different flu subtypes that infect humans, such as the presence of a broad-spectrum neutralizing HA epitope in H1 and H2 subtypes,[282,289-291] and the resistance of adults to infection following immunisation with live, attenuated pandemic trial vaccines, to which they are otherwise immunologically naïve.[292,293] The substantial selective pressure exerted by an immune population accelerates antigenic drift in B cell epitopes; the same may be true with T-cells, although their epitopes seem to be more highly conserved.[294]

Serum antibodies provide robust correlates of protection from flu infections and are critical to prevention and recovery from disease.[295-298] Antibodies to the HA, NA, NP and M proteins are produced following flu infection. The most critical to antigen neutralisation is that directed towards HA,[297,299-301] while antibodies to both HA and NA are fundamental in resistance to infections and in limiting disease severity.[295,296,302,303] HA antibodies function by interfering with virus receptor binding or fusion,[289,291,304,305] while NA antibodies provide antiviral protection by impeding the release and spread of progeny viruses.[306-310] The most often detected antibodies specific to HA in local nasal secretions, during primary infections, are IgA and IgM, but IgG is also present in low titres in systemic secretions.[311] IgA response is of both local and serum types. Following primary natural flu infection, local IgA levels remain for three to five months and there is local memory to flu antigens.[303] On the other hand, during reinfection, local IgA response is accompanied by serum IgA secretion, both of which are of equal strength.[303] The vertical transfer of maternal antibodies to infants whose mothers were vaccinated against flu during pregnancy best illustrates the importance of antibodies in host immunity to flu infections.[312-314]

T-cell responses regulated through both class I and class II major histocompatibility complex to flu infection are not well understood in humans and, therefore, most data derive from mouse model studies.[315] Class I ($CD8^+$) cytotoxic t-lymphocytes (CTLs) are detectable within six to fourteen days of either natural flu infection or live attenuated vaccination and persist until 21 days after infection.[316] They provide both strain-specific and cross-reactive protection to

FluA viruses of any subtype, but not to FluB viruses.[315,317-320] CTLs are associated with virus clearance from the respiratory tract following experimental inoculation with wild-type flu viruses, but not with decreased susceptibility to disease.[315] In comparison, class II ($CD4^+$) T-helper cells function by enhancing both B- and T-cell responses.[321-324] T-helper cells to the M or NP proteins augment antibody responses to HA antigens,[324] while also enabling the proliferation of CTLs.[321-323] They are also capable of virus cell lysis, through either the release of perforin or the antibody-complement complex.[325-328] Lymphoproliferation is another manner by which cell-mediated responses to flu infection may be ascertained, with lymphocyte blastogenesis evident from days three to six after infection and a return to baseline levels noticeable by day 28.[329]

8.3.2.4 Transmission

Human influenza viruses are not known to cause chronic or latent infections. Therefore, it is presupposed that they are sustained in the population by direct person-to-person transmission during acute infections.[140] It is suggested that aerosols may be the primary mechanism of virus transfer among individuals.[330-333] Self-inoculation with hands contaminated by fomites is also believed to be a potential source of flu spread in humans, particularly at temperatures ≥30˚C, when flu is inactivated in aerosols but not in fomites.[166,334,335] However, recent studies suggest that fomite spread is of little consequence, compared to bioaerosol transmission.[330,331]

Aside from person-to-person, avian-to-human transmission of flu viruses is also possible. The influenza A(H5N1) outbreak in Hong Kong in 1997 was the first time it was realised that avian flu viruses can be successfully transmitted to humans directly and, more importantly, that they have the potential to cause the next pandemic.[210-212,217,336,337] H5N1 re-emerged in Hong Kong in 2003, from where it continues its spread in poultry and humans worldwide. As of 25th July 2017, the total number of confirmed human H5N1 infections was 859, 53% of whom have died.[338] Other cases of avian-to-human flu transmissions include: in 2003, outbreaks of H7N7 in the Netherlands,[339,340] H9N2 in Hong Kong,[341,342] and H7N2 in New York;[343,344] in 2004, H7N3 in Canada [345,346] and H10N7 in Egypt;[347] in 2006,

H7N3 and H7N2 in the UK;[344,348] in 2008 and 2009, H9N2 drift strains in Hong Kong;[342] in 2013, H7N9 in China;[349,350] and in May 2016, H5N6 in China.[351] For the most part, these have caused few deaths and mild respiratory or conjunctival diseases in humans, compared to H5N1 infections.[140] The recent increase in detection of avian-to-human transmissions since 2003 may be due to more vigilance in surveillance systems, particularly in veterinary practices, more transparency and streamlined reporting methods, and increased close contact of humans with avian species.[140] However, none have so far caused a pandemic, primarily because they have yet to evolve a mechanism of sustained transmission among humans.[352]

Although flu easily spreads in the community, its primary mechanism of transmission in humans is not yet well understood due in large part to the lack of human models in natural transmission research.[12,353] Most studies in natural flu transmission use ferrets as animal models,[354,355] however, the associated costs and difficulties in their acquisition have led to the use of guinea pigs in more recent investigations, involving the contributions of aerosols, temperature and humidity in transmission.[356,357] There is also increasing research involving human challenge studies,[12,358] however, they are still in the inception stage and are mostly based on experimental infection of humans with varying doses of multifarious flu strains, and may therefore not be accurate models of what transpires in nature.

8.3.2.5 Prevention and Control

Vaccination is the best available method to prevent disease and severe clinical outcomes from flu virus infections.[166,359,360] It is strongly indicated for individuals at high-risk of serious flu complications, including pregnant women, children aged 6 months to 5 years, adults ≥65 years, chronically ill people, and healthcare workers.[166] Inactivated and live, attenuated seasonal vaccines have been in use for more than 60 years.[166] In healthy young adults, they are generally safe, well tolerated and reasonably immunogenic, provided the strain included in the vaccine is similar to the currently circulating drift strain.[166,361] In the elderly, a high-dose vaccine is used to prevent disease and reduce incidences of complications and deaths.[362-367] Until 2013, seasonal influenza vaccines came in

trivalent formulations, containing two FluA virus components (H1 and H3) and one FluB (either Yamagata or Victoria).[166,368] Since then, however, quadrivalent vaccines containing both FluB subtypes have been introduced.[369,370] FluC is traditionally not included in the annual vaccine, because infections occur less frequently than FluA and FluB.[166] There is currently a race to develop the first universal flu vaccine, to eliminate the guesswork that is sometimes necessary in determining the components of every seasonal flu vaccine for each of the northern and southern hemispheres.[371,372]

Antiviral treatments are available to individuals for whom flu disease cannot be prevented. There are currently four licensed antivirals: two M2 ion channel inhibitors (adamantanes) that are used against FluA - amantadine and rimantadine, and two neuraminidase protein inhibitors (NAI) that are effective against FluA and FluB - oseltamivir and zanamivir.[373,374] It was established in 2005 that almost 90% of circulating seasonal strains are resistant to adamantanes, however, susceptibility to NAIs remains high.[373] Furthermore, NAIs have been shown to not inhibit humoral antibody production and seroconversion, hence, protecting against reinfection.[375,376] Therefore, although adamantanes are no longer suitable for flu prophylaxis in humans, NAIs remain highly recommended for seasonal use and are stockpiled by affluent countries for future pandemics.[374]

Oseltamivir is administered as oral capsules in individuals aged ≥1 year old, while zanamivir is inhaled and licensed for use by people aged ≥7 years.[374] Oseltamivir improves survival rates in humans infected with H5N1 and[377-380] prevents severe disease and deaths among contacts of those infected with H7N7.[339,340,381,382] It is thus that, during the 2009 pandemic, oseltamivir was the antiviral that received emergency approval in the U.S. and Europe for use in infected children <1 year of age.[140] Other NAIs were also approved for emergency use in 2009, including IV peramivir and laninamivir, however, they remain unlicensed for use, except in Japan and Korea.[383]

Since very little is known about flu transmission, behavioural methods of controlling spread are directed towards aerosols and fomites and include social distancing, isolation, and handwashing.[139,166] Rapid diagnosis is also considered to

be beneficial, particularly because antivirals have the most beneficial effects when administered as soon as 48 hours after symptom onset.[139]

8.3.3 Coronaviruses (HCoV)

8.3.3.1 General Information

Coronaviruses belong to Coronavirinae, one of two subfamilies in *Coronaviridae*. Within this subfamily are the alpha, beta and gamma genera, which are differentiated according to their phylogenetic grouping. [384] Hosts of the alpha and beta genera are mostly mammals, including bats, cats, dogs, rats, horses, pigs, cows and humans. In contrast, gamma coronavirus hosts are primarily birds, with the exception of one strain that infects Beluga whales.[384] Coronaviruses were first identified in the 1930s as causative agents of transmissible respiratory and gastrointestinal infections,[385-387] as well as severe hepatic and neurologic diseases,[388,389] in animals. However, it was not until the 1960s that the importance of coronaviruses in humans (HCoV) was appreciated in common cold infections, upon identification of HCoV-229E and HCoV-OC43.[385,390,391] Subsequent decades witnessed the study of coronaviruses only as they related to the pathogenesis of contagious diseases in household pets and farm animals, until the emergence of SARS in China's Guangdong Province in November 2002.[384,392]

The SARS outbreak was caused by a novel coronavirus, comprehensive study of which profoundly broadened our understanding of coronaviruses in general.[393-395] In 2005, molecular surveillance methods identified two further strains that are highly infective in humans, HCoV-NL63 and HCoV-HKU1.[396-398] In 2012, the novel MERS-CoV emerged in Saudi Arabia.[399] It continues to spread around the globe, causing severe acute respiratory illnesses and death in many susceptible humans.[400-403]

Structurally, HCoVs are generally round in shape but their sizes may vary. The most remarkable family feature, for which they are named, is a fringe of club-shaped spikes that are spaced wide apart on their surface, making it appear as though they have a surrounding solar corona (latin, for crown).[384,392] They have a

bi-layered envelope containing the spike (S), membrane (M) and envelope (E) surface proteins and a RNP core that contains the nucleocapsid (N), which surrounds a helical positive-stranded RNA genome. A hemagglutinin-esterase protein (HE) is found on the surface of HCoV-HKU1, which functions in attachment to host cells and rapid transport through extracellular membranes.[384,392] A schematic of SARS-CoV that indicates the locations of its RNA and the S, M, E, and N proteins are shown in figure 4 below.

8.3.3.2 Epidemiology

Four out of the six known HCoVs are endemic in the human population.[404,405] Those in the *Alphacoronavirus* genus (group 1) include HCoV-229E and HCoV-NL63, while HCoV-OC43 and HCoV-HKU1 belong to the *Betacoronavirus* genus (group 2).[404,405] All four strains have a worldwide prevalence that has been traced as far back as the 11th century although, remarkably, NL63 and HKU1 were only identified less than a decade ago. Therefore, most epidemiological data on endemic HCoVs are derived from studies on OC43 and 229E. [404,405]

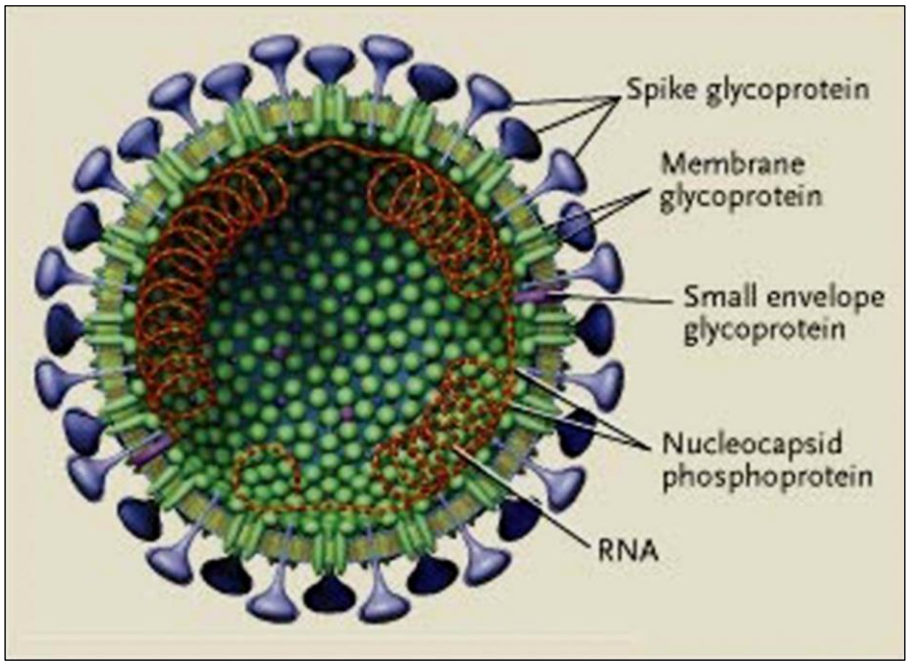

Figure 4 SARS-CoV Schematic [d]

[d] Taken from Drazen, 2003[406]

HCoV-OC43 and 229E are responsible for between 1% and 34% of all URTI in all age groups annually.[407] These rates vary appreciably due to differences in annual incidence, seasonality, age at infection and method of pathogen detection.[407] Incidence rates are highest every two to four years, during winter and spring in temperate regions, among younger children, and with PCR diagnostic methods.[408-411] Neonates and the elderly are at increased risk of severe LRTI, particularly those with underlying COPD and diseases that require ICU admissions.[412-414] In comparison, NL63 and HKU1 are implicated in approximately 10% of generally mild URTI infections. However, NL63 is also a common pathogen in childhood croup and HKU1 can cause severe pneumonia in the elderly.[398,415,416] HKU1 is not well studied due to difficulties in strain isolation *in vitro*,[398] although it seems to have the same seasonality as OC43 and 229E. In contrast, NL63 tends to be active during the summer and autumn in sub-tropical regions.[417,418]

The other two HCoVs, SARS-CoV and MERS-CoV, are zoonotic strains belonging to the *Betacoronavirus* genus that cause epidemics in humans.[419,420] SARS-CoV did not circulate in the human population extensively prior to the outbreaks during 2002 to 2003,[419,420] and was primarily seroprevalent in asymptomatic workers in wild animal wet markets in China.[421] However, in the weeks following the initial outbreak, it rapidly infected almost 8000 people in 29 countries within five continents, killing approximately 10%.[395] On the other hand, MERS-CoV was first reported in a 60-year-old male from the Kingdom of Saudi Arabia (KSA) in September 2012.[422] Retrospective analysis suggests, however, that the index case was actually one of 11 infected during a hospital outbreak in Jordan, in April 2012.[422,423] As of 21st Sept. 2017, 2081 confirmed cases of MERS-CoV were reported from 27 countries, 35% of which resulted in death.[403] Most at-risk of infection and death are males aged ≥60 years, while only approximately 1% of children <14 years become infected.[422] More than 85% of the cases are in Saudi Arabia, and those cases reported from countries outside the Arabian Peninsula may have been exported from the region. To date, the outbreak in the Republic of Korea from June to October 2015 remains the largest export of MERS-CoV infections from the Middle East.[424,425]

8.3.3.3 Infection and Immunity

Clinical patterns of HCoV disease differ according to whether infection is due to endemic or epidemic strains.[384] Primary infections with endemic OC43, 229E, NL63 and HKU1 occur early in childhood, while reinfections with the same serotype occur in the immediate months following primary infection and recur throughout life.[407,409,426,427] Serum antibodies are detectable in approximately 50% of children and 80% of adults,[409,428] but cross-protective antibodies to infection with other HCoV strains are not evident.[392] Endemic HCoVs primarily cause upper respiratory symptoms in children and adults, such as rhinorrhoea, pharyngitis and cough, often accompanied by systemic signs that include fever, headache, malaise and chills.[429,430] Infection in neonates may manifest as gastroenteritis.[431-433] The incubation period is generally two days and disease peaks within three to four days of infection, but symptoms may last up to 18 days.[429,430,434] Clinical manifestations are consistent with damage to ciliated epithelial cells, and excessive chemokine and cytokine production, within the nasal cavity.[435] Approximately 30% of URTI are asymptomatic.[429,430]

Infections with endemic HCoVs may also cause LRTI, which result in hospitalisations in approximately 8% of children and 5% of adults.[417,426,427,436-438] Wheezing and asthma exacerbations may also be associated with any of the endemic strains,[397,407,426,439] however, croup in children < 3 years has, so far, only been linked with NL63 infection.[440] Note that, due to the number of asymptomatic infections, similarities in disease presentation with other respiratory viruses, and little data on the more recently discovered NL63 and HKU1, direct correlations between HCoV infections and acute respiratory diseases must be made with caution until more definitive data become available.[441,442]

By contrast, infections with epidemic SARS-CoV and MERS-CoV have generally resulted in severe LRTI that required ICU admissions and mechanical ventilation in older populations with underlying co-morbidities.[395,403,423,443] In the SARS outbreak, disease severity was directly proportional to age at infection and underlying health conditions, with approximately 50% of cases over the age of 60 years and mortalities not evident in those aged <24 years, but common in those

otherwise ill.[443-446] Incubation periods ranged from two to fourteen days and symptom onset was four to seven days later.[384]

Characteristic of SARS infection were systemic symptoms that included fever, myalgia and malaise, followed by non-productive cough and shortness of breath (SOB) in most, or viral pneumonia in others.[395,443-446] Failure to recover from initial symptoms often resulted in disease progression to acute respiratory distress syndrome (ARDS), followed by death within weeks or months after the onset of illness.[447,448] Meanwhile, those able to resolve initial symptoms fully recovered, but their lung functions either remained impaired or took several months to return to baseline levels.[449,450] Furthermore, there were higher than expected numbers of SARS survivors who developed neurologic or psychiatric impediments, particularly in those who had a more severe respiratory disease and higher degree of steroid therapy, suggesting the occurrence of CNS infiltration by SARS-CoV.[451-454] Asymptomatic and mild infections only occurred in <1%, based on studies involving HCWs, who were the most at risk of infection in the healthy young adult population.[455-457] Note that the clinical manifestations of SARS were not pathognomonic and most data were extrapolated from those with either a familial or nosocomial cluster history of SARS infection. However, some pathologies were more common to SARS cases than to those infected with other respiratory pathogens.[443-446]

Symptoms associated with MERS-CoV may be either mild or severe.[403,458] It was initially suggested that asymptomatic infection also occurs however, upon closer investigation, it was determined that >75% of these cases were misreported.[459] Typical infections include a prodromal period of fever and gastroenteritis that, when almost resolved, is followed by a more severe respiratory syndrome with cough and SOB.[422,460,461] Pneumonia often follows, but not all those infected develop the condition.[403] Disease may progress to severe ARDS, requiring ICU hospitalisation and mechanical ventilation.[403] Death occurs in 36%, often in older people, but particularly in those immunocompromised or with co-morbidities such as cancer, chronic CPD and diabetes.[403] The incubation period ranges from two to sixteen days,[462-464] and time-to-death following severe disease are between five

and 27 days.[41,462] Since MERS-CoV infection is very much still evolving at present, data on infection patterns may be likely to change.

Host immune response following HCoV infection often begins with the production of type I IFN. PDCs are the foremost sources of IFN-α and IFN-β that are induced by HCoV, however, macrophages may also be activated to express type I IFN.[465-468] Expression of IFN by pDCs are regulated through TLR-7- and IRF-7-dependent signalling pathways, which were first elucidated in mice lacking receptors for IFN-α/β.[466,469] Although the relevance of IFN in host immune response to HCoV infections is well established, it remains unclear which specific protein activated by IFN is most essential to immunity.[469] It is suggested that ribonuclease L (RNaseL) may be that protein, based on its critical role in the immunity of other host species to their respective infecting neurotropic CoV strains. However, more data are needed to establish the importance of RNaseL in non-neurotropic CoV infections in humans.[470]

Subsequent to type I IFN production, T-cells and macrophages are transported to the sites of infection to eliminate HCoV, through pro-inflammatory host responses that include the activation of cytokines, chemokines and their respective receptors.[435] In infections of the CNS, neutrophils or their associated CXCL1 and CXCL2 chemoattractants are essential in infiltrating the blood-brain barrier, without which, the pathology of disease will be more severe.[471] Crucial to virus inactivation and clearance are T-cell responses of both CD4 and CD8,[472,473] whose epitopes are located on the S, M and N proteins.[474-477] Neutralizing anti-HCoV antibodies prevent virus reactivation and have been associated with symptom resolution during the SARS-CoV outbreak.[465,478] However, cross-protection among different virus strains is not evident.[392] Once HCoV has been neutralised, anti-inflammatory mechanisms such as Foxp3 expression of regulatory CD4 T-cells and IL-10 stimulation by CD4 and CD8 cells, may be activated to prevent a pathogenic immune response in the host.[479-481]

8.3.3.4 Transmission

Increased incidence in early childhood and outbreak patterns suggests that HCoVs are extremely transmissible. This may be through the faecal-oral route, or the

infection of the nasal epithelium by three mechanisms: direct large droplet contact, indirect aerosol inhalation, or self-inoculation following contact with contaminated surfaces.[407,409,426,427,435] However, in contrast to the epidemic SARS- and MERS-CoV, very little is known about the mechanisms of endemic HCoV transmission. Hence, the remainder of this section focuses on the transmission of epidemic HCoVs.

The global spread of SARS-CoV was very much due to human-to-human transmission.[443] Household and hospital settings experienced the most infections, generally because virus transfer transpired only after illness had started and was most efficient when a person was ill enough to be highly contagious and to be admitted to hospital.[395,482] The overall basic reproduction number (R_0) of SARS was approximately 2-4, even though most of those infected did not transmit infection.[482-484] 'Super-spreading' events, where R_0 was disproportionately higher than normal, occurred on a few occasions, primarily due to a combination of environmental factors and either host elevated virus titre or efficient droplet aerosolisation.[482,484]

Large droplet contact is believed to be the primary method of SARS transmission, although aerosols from mechanical ventilation devices may have also promoted airborne virus transfer.[485-487] Furthermore, aerosolised faecal matter from sewage is believed to have caused the largest super-spreading event that took place in an apartment block in Hong Kong.[488] It was presumed that fomite transmission may also have occurred, however, this was based only on the finding that SARS had a longer survival period on surfaces than HCoV-229E.[489,490]

In contrast to SARS, MERS-CoV inter-species transmission to humans is being attributed to a strain that naturally infects adult dromedary camels only found in the KSA, the Arabian Peninsula and parts of Africa, from where camels in the KSA may have been imported.[403,422,423,491] Although direct camel-to-human transmission occasionally occurs, the exact role of camels and the precise mechanism(s) of transmission have yet to be elucidated.[403] Supportive evidence for camel-to-human transmission due to contact with droplet nuclei is the detection of high titre MERS-CoV RNA in the upper respiratory tract and lungs of

infected camels and in air samples collected from their barns.[492,493] However, these do not account for cases that have had no direct contact with either infected camel or another human case.[494]

Similar to SARS-CoV, most human infections with MERS-CoV are attributed to person-to-person transmission, although MERS-CoV is not as easily transmissible and virus spread is unsustainable (R_0=1).[42,403,495-498] Close contact is presumed to be the primary mode of transmission, as evidenced by cases in households and among hospital inpatients and HCWs.[458,463,499,500] Also similar to SARS, MERS-CoV transmission rates are greatest in HCWs, at approximately 20%, particularly during times when strict adherence to infection control policies is disregarded.[491] At the same time, an approximate decrease of only 7% in airborne viability at room temperature and low relative humidity, compared to 95% decrease in FluA, establishes the possibility of aerosol transmission.[422] However, since the MERS-CoV outbreak remains an evolving situation, future findings may be contradictory, not least because of challenges experienced in determining contacts, diagnostic testing in communities, and defining what constitutes a case.[422,501] With the amount of active research on MERS-CoV at present, differences in severity of disease and outcome of infection between camel-to-human and human-to-human transmissions may, perhaps, soon be established.[501]

8.3.3.5 Prevention and Control

The only available vaccines for HCoV are those specific for SARS-CoV.[502,503] The formulations include inactivated whole virus, live virus vectors that express either a single or a recombinant protein, and DNA vaccines.[502,503] Production of these types of vaccines is proving problematic, because many cannot surpass the short-lived immune responses induced in natural infections, thereby leaving patients at risk of reinfection and illness.[504] Moreover, these vaccines may not provide comparable defences against all antigenic variants, and genetic recombination of vaccine strains with wild types may have deleterious implications in currently circulating HCoVs.[505,506] Finally, antibody-dependent enhancement of disease (ADE) may occur during natural infections, following immunisations with vaccines

that express the S glycoprotein.[507] Strategies are in development to overcome the challenges with SARS-CoV vaccines, whether real or presumed.[508-511]

There are also no antiviral therapies for HCoV infections. At the height of the SARS-CoV outbreak, supportive therapies were used to help manage the situation.[384] These include treatments with ribavirin, which was used to inactivate the virus, and high dosage corticosteroids, which were used to attenuate immune pathogenicities.[512] As the outbreak progressed, IFN-α, a SARS convalescent plasma immunoglobulin and two licensed protease inhibitors commonly used to manage infections with human immunodeficiency virus (HIV), were prescribed in varying degrees, in the belief that they might inhibit further SARS-CoV propagation.[513-518] However, these desperate measures were not found to be protective in patients.[512]

Due to the absence of effective vaccines and antivirals to HCoVs, strict adherence to behavioural strategies are in place.[384] These include vigorous public health surveillance systems of sharing relevant information for rapid identification of cases and contacts, and isolation and infection control methods that minimize human-to-human and super-spreader transmission events.[455-457] To prevent cases of laboratory-acquired HCoV infection and subsequent community transmissions, visibility in numbers of episodes, timely identification of cases and contacts, and careful attention to good clinical laboratory practices are recommended.[403,491,519,520] However, without knowing the precise mechanisms of infection and transmission, it cannot be determined how effective these strategies are.

8.3.4 Adenoviruses (HAdV)

8.3.4.1 General Information

Adenoviridae is a family of viruses that have only been identified in vertebrates. Within these, its host range is diverse, infecting all populations from fish to mammals.[521,522] Its members are divided into four genera: *Mastadenovirus, Aviadenovirus, Atadenovirus and Siadenovirus.* Human adenoviruses belong to the first genus, where there are seven species and approximately 60 types.[521-523] HAdV

species A through G are distinguishable by serology, hemagglutination, CPE and DNA sequence, as well as by their oncogenic properties in rodents.[521,522,524] HAdVs are capable of causing a spectrum of diseases, and each species can be identified further according to the pathology of disease they cause in humans.[521,522] Table 1 below lists the HAdV species and types associated with particular clinical syndromes and the commonly affected populations.

Two different groups working on establishing the aetiology of acute respiratory diseases first identified adenoviruses in humans in 1953. Hilleman and Werner's (1954) group reported the recovery of an unidentified virus from throat samples of a U.S. Army recruit in Fort Wood, Missouri, who had atypical pneumonia during a respiratory epidemic between 1952 and 1953.[521,524,525] At around the same time, Rowe and colleagues (1953), in their attempts to identify tissue culture cell lines using tonsils and adenoidal tissue removed in routine paediatric surgeries, observed a transmissible pathogen that spontaneously degraded tissue culture cells.[522,524,526] Subsequent immunological comparability tests were performed, which led to the conclusion that the pathogens were similar and were thus named after the tissue from which they were first recovered.[524,527] Since their discovery, it has been established that, similar to the newly identified hMPV, HAdVs are also not emergent viruses. They can be traced as far back as World War II, during an acute respiratory disease outbreak in another U.S. Army facility in Fort Bragg, North Carolina.[524]

Table 1 Clinical Syndromes Associated with HAdV Infections[e]

Clinical syndrome	Adenovirus species	Known types	Population at risk
Endemic respiratory	B, C	1, 2, 3, 5, 6, 7	Infants, children
Epidemic respiratory	B, C, D	5, 7, 55, 56	Children
Acute respiratory disease	B, E	3, 4, 7, 14, 21	Military recruits
Pharyngoconjunctival fever	B, C, E	1, 3, 4, 7, 14	School-age children, young adults
Keratoconjunctivitis	B, D	8, 11, 19, 37, 53, 54, 56	All age groups
Hemorrhagic cystitis	B	11, 21, 34, 35	Immunocompromised patients, children
Gastroenteritis	F, G	40, 41, 52	Children, immunocompromised patients
Other syndromes	A, B, C, E, D	2, 4, 7, 12, 19, 32, 37	Children, adults
Immune deficiency	B, D	34, 35, 43-49	Transplant recipients, persons with acquired immunodeficiency syndrome, and immunocompromised patients

[e] Taken from Gray, 2013[528]

Animal studies established the association of HAdVs with oncogenesis in rodents, which was when their wide-ranging applicability in research began to be appreciated.[521] In cell culture, HAdVs propagate well and set in motion observable simultaneous reactions that aid in the understanding of virus life cycle and replication.[521] In molecular research, their genomes can be manipulated without much difficulty, so that the roles of mutations in disease pathology are better understood. [521,529,530] In gene therapy, HAdV contributions are most recognised, due to their ability to splice messenger RNA, which makes them excellent virus vectors for treating disorders by genetic manipulation.[521,529,530]

Structurally, HAdVs are non-enveloped and icosahedral in shape. A capsid shell surrounds the virus core, which contains a linear, double-stranded DNA.[521,524] There are three major capsid proteins, which include the hexon, fiber and penton base. These proteins confer viral surface antigenicity and are therefore, targets for host neutralising antibodies.[521,522,524]

8.3.4.2 Epidemiology

Worldwide, HAdV infections can be endemic, sporadic or epidemic. The nature of the infection is largely dependent on the strain of the virus and the age of the host.[522,524,531,532] There is also a distinct difference between the epidemiology of HAdV infections among military personnel and civilian populations.[524,533] However, this may just be because most available data came from longitudinal non-military surveillance systems. These include the New York and Seattle Watch studies by Fox et al. (1969, 1977) during 1961 to 1969[522,531,532] and the Manchester, England surveillance by Cooper *et al*. (2000) from 1982 to 1996.[522,534] On the other hand, military studies were based on outbreaks in incoming recruits that occurred during seasons when vaccines were not available.[525,535]

Global HAdV infections are associated with approximately 8% of viral diseases that are of clinical importance.[522] Disease generally occurs in 3% of the civilian population, but could be as high as 5% in children and 10% if associated pyrexia alone is measured.[531,532] The serotypes most often associated with disease belong to species HAdV-B, C and F.[522] In infants, HAdV-C and F are more prominent, while HAdV-B and E are more common in adult infections.[522,534] Approximately 75% of

symptomatic HAdV infections result in concomitant antibody production and there are distinct serum antibodies in children and adults. Serum antibodies to HAdV-C in children are high and present in 40% to 60% of infections, while antibodies to types in HAdV-B and E in adults are low.[531]

The epidemic nature of HAdVs was mostly studied during the outbreak of acute respiratory disease (ARD) in U.S. military personnel during World War II.[533,536,537] In the autumn and winter, incoming recruits were infected with types of either HAdV-B or HAdV-E. Since senior staff and other students were not infected, it was presupposed that social factors, such as fatigue and over-crowding, may be associated with epidemic HAdV.[522,533,536-540] Current findings suggest that approximately 80% of incoming recruits become ill from HAdV infections.[522] Symptomatic shedding lasts for only a short duration of up to four days, nevertheless, 20% to 40% end up in hospital with severe disease.[541] Outbreaks of HAdVs have also been recently observed in civilian hospitals, where they have the tendency to be fatal, highly infectious and have new tissue tropisms.[523,542,543] Genome analyses suggest that these strains are either re-emerging viruses that have developed virulence, or recombinant strains that are extremely pathogenic.[542,543]

HAdV infections also affect the eyes and cause pharyngoconjunctival fevers (PCF) and epidemics of keratoconjunctivitis (EKC). Strains in species HAdV-B and C are most often associated with PCF, but several other strains in HAdV-E are also implicated in eye infections.[524] Strains in HAdV-B and E are primarily identified in swimming pool-associated eye infections.[544] Geographically, serotype 4 (HAdV-E) is prevalent in Asian eye infections, but not eye diseases in western countries.[524] On the other hand, EKC in developed countries are most often associated with three strains in HAdV-D, and are generally due to nosocomial outbreaks,[524,545] while in poorer countries, these same strains are more likely to be endemic.[524]

In enteric HAdV infections, infant gastroenteritis is more likely to be caused by strains belonging to HAdV-F.[524] These strains tend to be endemic worldwide and are spread through the faecal-oral route. All other HAdV species, with the

exception of HAdV-E, are also capable of causing acute gastrointestinal infections.[524,546,547]

8.3.4.3 Infection and Immunity

In humans, HAdV infections generally occur in childhood. Most infections tend to be either subclinical or mild and self-limiting; however, both still induce strain-specific immune responses.[522,524] Serotypes have distinct cellular affinities and clinical pathologies, while genotypes are mostly associated with virulence and disease severities.[524,542,548-550] HAdVs most frequently infect the respiratory and gastrointestinal tracts, as well as the eyes. Less often, they infect the urinary tract and organs such as the liver, pancreas and heart, and occasionally, even the CNS.[522,524,551] This chapter focuses only on respiratory infections.

In children, HAdV respiratory infections are generally endemic. Approximately 10% of URTI in children <5 years old are associated with virus, usually from species HAdV-B and C.[2,522,552] Symptoms are generally coryzal and include nasal congestion, runny nose, sneezing, pharyngitis and post-nasal drip. Systemic signs also often develop and may include headache, fever, chills, muscle aches and malaise.[522] On occasion, exudative tonsillitis can occur, which may be inaccurately diagnosed as *Streptococcus* A infection. Conjunctivitis may also sometimes develop with respiratory disease, and the illness is then referred to as PCF.[522] HAdVs also cause approximately 10% of LRTI in children hospitalised for pneumonia.[552] Most children recover, but mortality can be higher than 16% during epidemics, particularly in neonates.[43] Further, lung damage may develop, predisposing one to long-term susceptibilities to respiratory infections, which may not be observed until years after primary infection.[2,43,552] Severe cases of LRTI are most often associated with Ad7 of species HAdV-B, however, four other strains from HAdV-B, C and D are also implicated in morbidity and mortality in children worldwide.[43,552-556] Virus clearance through the respiratory tract can take between one and twelve weeks, however, excretion through stools could take months, and may be the reason for its endemic nature in this population.[45,524]

In adults, mild acute respiratory infections are mostly associated with strains belonging to HAdV-E and to AdV-B1, sub-species of HAdV-B. In contrast, AdV-B2 is

more often linked to epidemics, mortalities and severe morbidities, which may include urinary tract infections and opportunistic diseases in the immunocompromised.[557] Presenting symptoms generally include fever and cough that result in hospitalisation, supplemental oxygen therapy and ICU admittance in more than 50%, and death in approximately 20%.[44,548,557-561]

In new military recruits, syndromes of adenovirus-associated ARD (AARD) are similar to those in children with mild respiratory HAdV infection, mostly due to HAdV-E.[562] HAdV-B infections occur less often, but can be deleterious in epidemic situations.[562] Strains belonging to sub-species HAdV-B1 and HAdV-B2 that cause increased morbidities and mortalities in adult civilians have similar pathologies in military personnel, particularly the newly emerged Ad14 strain.[557,562,563]

HAdVs are also associated with approximately 23% of patients with pertussis-like syndromes.[564] In fatal cases of whooping cough, strain Ad5 is often the pathogen identified.[564-568] However, when HAdVs are identified in whooping cough syndromes, *Bordetella pertussis,* the causative agent of pertussis, is also usually present.[566] Therefore, it remains to be determined which is the cause and the effect.[569]

Host innate immunity to HAdV includes structural and chemical antiviral responses to infection. Surface sialic acids bind virus species that have receptors for this structural compound, thereby inhibiting infection.[570,571] Meanwhile, chemical antivirals that directly neutralise HAdV include defensins that block the release of progeny viruses, particularly α-defensin HNP1, which is found in leukocytes and natural killer cells.[572-574] Hosts also produce IFNs that function in apoptosis of infected cells.[524]

Cellular immune responses induce virus elimination by alveolar macrophages in the lungs and by Kupfer cells in the liver, through the secretion of pro-inflammatory cytokines that include TNFs, IL-6, IL-8 and IL-12.[575,576] In children, particularly stem cell transplant (SCT) patients, there is an associative cytokine storm of IL-6, IL-8 and TNFs, regardless of whether HAdV infections are localised or invasive.[577] The T-cells CD4 and CD8 are also critical in virus clearance, both of which are associated with recognition of conserved antigen motifs across several

serotypes.[578,579] Almost all adults have $CD4^+$ and $CD8^+$ T-cells specific for HAdVs, which are cross-reactive among serotype hexon protein epitopes.[522] $CD4^+$ cells also provide prolonged host immune responses to HAdV.[580] In immunosuppressed adult SCT patients, a combined $CD4^+$ and $CD8^+$ T-cell response against the HAdV capsid hexon is critical to the resolution of viremia.[581] Furthermore, CTLs prevent graft-versus-host-disease when infusions of donor lymphocytes are used as HAdV prophylaxis in SCT recipients.[578,579,581-583]

Humoral responses to primary infections include both species- and type-specific antibodies to HAdV. Although species-specific serum antibodies do not overcome virus infectivity, type-specific antibodies can neutralise epitopes of the capsid penton, fibre and hexon proteins.[531] Neutralisation provides protection against symptom development and reinfection with the same serotype, which was the basis for vaccine production aimed at military personnel.[531] However, although antibodies are protective, hosts remain virus carriers during infection. Therefore, following a successful humoral response, carriers continue to sporadically shed virus for months, particularly in stools.[531]

8.3.4.4 Transmission

HAdVs are believed to be transmitted both directly, through contact with individuals shedding virus or through the faecal-oral route and indirectly, by inhalation of aerosols, self-inoculation after contact with fomites, or ingestion of contaminated water.[524] Children are infected primarily through the faecal-oral route, based on household studies where at least one member had gastrointestinal HAdV.[584] However, given that HAdVs survive longer in the intestines than in the respiratory tract,[522,531] and stool shedding occurs sporadically up to a period in excess of 900 days,[45] it is highly likely that most household infections would be through the faecal-oral route.[584] In contrast, a study on incarcerated adult males by Couch, *et al.* (1966) suggests that HAdV respiratory disease is brought about by inhaled aerosolised particles and not by infection through the mouth, nose or intestines.[585]

Fomite transmission is thought to be particularly efficient, due to findings that HAdVs survive for long periods at room temperature, on account of its resistance

to mechanical disruption and chemical agents.[522] Strains that cause EKC, for example, can be recovered from nonporous surfaces for as long as 35 days, despite exposure to hot water or disinfectants.[586,587] However, fomite transmission was ascertained through *in vitro* experiments using either desiccated viruses or virus suspensions of increased titres [586,587] that may not be representative of what occurs in nature. Finally, although outbreaks of conjunctivitis have been correlated with exposure to HAdV in inadequately chlorinated swimming pool water, the virus has only ever been isolated from patient conjunctiva and not yet from the water in the swimming pools where infections are believed to have originated.[522]

8.3.4.5 Prevention and Control

A vaccine to prevent military AARD in military recruits was developed in the 1960s. A combined effort between the U.S. Department of Defense and National Institutes of Health led to the production of enteric-coated, oral vaccine tablets using live Ad4 and Ad7 strains.[361,588] These vaccines were designed to bypass the respiratory tract so that they replicate only in the intestines once the coating has dissolved. [589] They were successful in controlling HAdV infections in the military for more than 25 years, but were orphaned in 1996, when manufacturing ceased due to economic reasons. This interruption in immunisation resulted in the immediate return to pre-vaccine levels of HAdV epidemics in the military.[361,535,590] In March 2011, oral vaccine tablets against Ad4 and Ad7 were again licensed for use in military personnel aged between 17 and 50 years, with a reported vaccine efficacy of approximately 99%.[588] These vaccines are not licensed for use in children or civilian populations due to concerns regarding incomplete virus attenuation, which may result in symptomatic transmission to susceptible contacts.[540,591,592]

There are no licensed antivirals for HAdV infections. However, clinical trials on cidofovir, ganciclovir, and ribavirin are showing promise in transplant patients,[593-595] for whom early treatment provides the best outcome for antiviral therapies.[596] Current active research in alternative therapies that include the use of donor lymphocyte infusions and intravenous (IV) immunoglobulins are also reporting

encouraging results, however more data are needed for licensure to be granted.[597,598]

In the understanding that the capsular structure of HAdVs enable them to survive for long periods in the environment,[599,600] the use of germicidals are currently being investigated for the disinfection of ophthalmic equipment and environmental surfaces, with varying degrees of success.[587] Also, long-wavelength ultraviolet light (UV) and treatment with chlorine derivatives or ozone are used in the inactivation of enteric HAdVs in surface and groundwater supplies.[601-604] A combination of UV and other virus inactivation systems are also in development for use in blood and blood products.[605-608] However, perhaps successful interventions remain elusive, because of the lack of understanding of the natural transmission of HAdVs.

8.3.5 Paramyxoviruses

Paramyxoviridae is another virus family that is of exceptional importance to medical virology, in having for its members several of the most prevalent disease pathogens known in humans and animals.[609] Pathogens include the measles virus, which is the most contagious known;[609] RSV, PIV, hMPV, and mumps virus, which are the most commonly identified;[609] Newcastle disease virus, a blight in the poultry industry;[609] and the recently identified Nipah and Hendra viruses, that so far only cause fatal infections.[609] There are two branches to the family, the subfamilies *Paramyxovirinae*, which has seven genera and *Pneumovirinae,* with two genera.[609] Taxonomic classification is determined according to capsid morphology, antigenic cross-reactivity, neuraminidase function, and genetic processes.[609] Of specific importance to this study are RSV and hMPV of the *Pneumovirinae s*ubfamily and PIV of the *Paramyxovirinae* subfamily, which are reviewed more closely in subsequent sections.

8.3.5.1 Respiratory Syncytial Virus (RSV)

8.3.5.1.1 General Information

Human RSV was first identified in 1955, when a laboratory chimpanzee developed respiratory symptoms similar to that of human with a cold.[610-612] Its name was derived from giant multinucleated cells (syncytia) that were formed when the virus was isolated in cell culture.[612] RSV was later established as the foremost cause of LRTI in infants and children worldwide.[611] Its clinical impact in severe respiratory disease in the elderly and immunocompromised was recognised soon after, thereby making it a prime candidate for broad age-range vaccine development.[361,613-615] Thus, RSV was traditionally considered to be a virus that caused severe infections at opposite ends of the age spectrum.[32] More recently, however, it has been suggested that RSV infection in healthy young adults may not be as inconsequential as initially understood, and that it may be second only to influenza in severe clinical manifestations of respiratory disease.[616]

The RSV genome consists of a single-stranded, negative-sense, enveloped RNA with 10 genes that code for 11 proteins.[612] The virus is made up of a nucleocapsid core of replicase proteins (N, P and L) and a non-segmented viral RNA that is enveloped in a lipid bilayer containing three surface transmembrane glycoproteins (G, F and SH).[611,612] These glycoproteins are involved in virus entry into and infection of host cells, making them primary targets for host antibody neutralisation.[611,612] Inside the lipid bilayer is also found the matrix protein, which may be involved in the assembly and packaging of virion components at the plasma membrane, prior to progeny virus budding.[611] Figure 5 below is a schematic of RSV identifying the lipid bilayer, non-segmented RNA and the replicase, transmembrane and M proteins.

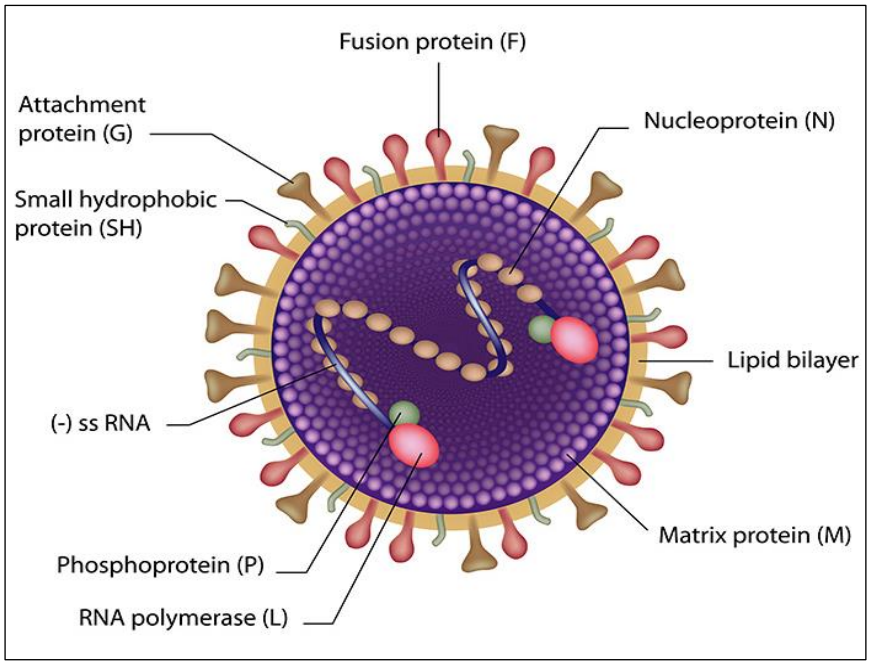

Figure 5 Schematic of RSV Virion[f]

RSV has one serotype with two antigenic subgroups, RSVA (64 strains) and RSVB (6 strains). The subgroups differ from each other the most in their G surface protein, with only a 53% similarity in amino acid sequences.[617] Table 2 below shows the per cent amino acid sequence similarities between structural and functional proteins of RSVA and RSVB, and among other paramyxoviruses. RSVA is more prevalent globally. However, both subgroups tend to circulate at the same time during epidemics and dominance of either subgroup shifts every 1 to 2 years.[611,612,618,619] Research into appropriate, immunogenic and safe vaccine candidates continue to be impeded by virus lability and inability to grow in cell culture.[611]

[f] Taken from Collins and Karron, 2013[611]

Table 2 Amino Acid % Similarities Between RSVA and RSVB and Among Other Paramyxoviruses[g]

Viruses compared		% Amino acid sequence identity for the indicated protein										
		NS1	NS2	N	P	M	SH	G	F	M2-1	M2-2	L
HRSV-A versus:	HRSV-B	87	92	96	91	91	76	53	89	92	72	93
	BRSV	69	84	93	81	89	38	30	81	80	42	84
	PVM	16	20	60	33	42	23	12	43	43	10	53
	HMPV-A	–[b]	–[b]	42	35	38	23	15	33	36	17	45
	AMPV-A	–[b]	–[b]	41	32	38	19	16	35	37	12	43
HMPV-A versus:	HMPV-B	–[b]	–[b]	96	85	97	59	37	95	96	89	94
	AMPV-C	–[b]	–[b]	88	68	87	24	23	81	83	56	80
	AMPV-A	–[b]	–[b]	70	58	77	20	12	68	73	25	64
	AMPV-B	–[b]	–[b]	69	53	76	20	13	67	71	27	ND[c]

HRSV, human respiratory syncytial virus; BRSV, bovine RSV; PVM, pneumovirus of mice; HMPV, human metapneumovirus; AMPV, avian metapneumovirus.
[a]Viruses are listed in order of decreasing relatedness to HRSV-A or HMPV-A.
[b]Does not encode NS1 or NS2.
[c]ND, not done.

8.3.5.1.2 Epidemiology

RSV epidemics occur annually. In temperate regions, yearlong persistence has been documented, but outbreaks more often occur for a period of four to five months between winter and early spring.[611,620] Globally, RSV epidemics are more common in moderate humidity and at temperatures either between 2˚C and 6˚C, or between 24˚C and 30˚C.[621] Annual outbreaks are suggested to be localised, rather than regionally or nationally widespread, and are characterised by independent introduction and spread of each virus strain.[361,612] This is particularly noticeable in nosocomial outbreaks, wherein multiple strains circulating in the community are introduced into patient wards on several occasions.[361]

Serology provides the most sensitivity in the diagnosis of RSV infection.[361] In comparison to this method, virus culture and antigen detection methods identify 50%, while molecular-based assays, such as PCR, can identify up to 75%.[361] Hence, the epidemiological data provided below are likely to be underestimated, due to limitations in sensitivities of the diverse detection methods used in studies.

8.3.5.1.2.1 Infants and Children

Worldwide, RSV infection in children < 5 years of age results in an upsurge in hospitalisations and deaths due to bronchiolitis and pneumonia each year.[361]

[g] Taken from Collins and Karron, 2013[611]

Infection leading to LRTI is estimated to be 34 million annually, which makes up 22% of all cases of LRTI in this population. Hospitalisation occurs in approximately 4 million and death in hundreds of thousands.[611,622,623] Increased medical consultations for RSV-related illnesses not leading to hospitalisations are also typical in children aged two to five years, with estimates ranging from 2.1 million to 4.2 million annually.[611,624,625] Symptomatic reinfection occurs at least twice more between the ages of two and three years, although, symptoms tend to be less severe.[611,626,627]

RSV infection is most common in infants than in older children. Approximately 70% of infants acquire the virus before their first birthday, and nearly all of them would have had the disease by the age of two years.[611,626-628] Primary infection results in the development of acute respiratory symptoms, with 25% to 40% progressing to LRTI.[611,626] Hospitalisations due to LRTI is most pronounced in those aged between six weeks and six months.[611,624]

RSV hospitalisations occur in 50% to 70% of otherwise healthy, full-term birth children.[629] However, day care attendance, paediatric hospitalisation during an RSV epidemic, and household residence of other children < 5 years of age increase paediatric exposure to RSV, putting them at high risk of infection and severe disease.[630,631] Other risk factors in paediatric RSV are primary infection, young age, premature birth < 28 weeks' gestation, inadequate maternal antibody titres, and underlying congenital and immune disorders.[611,630-634] Asthma or atopy, tobacco smoke, male gender and low socioeconomic status are also associated with predisposition to RSV disease.[611]

8.3.5.1.2.2 Elderly and Other High-Risk Populations

RSV is generally associated with morbidity and mortality in the elderly.[614] In developed countries, RSV-associated mortality is more pronounced in the elderly, at 78%.[635] This is more than likely due to underlying pneumonia, congestive heart failure (CHF), or exacerbations of asthma and COPD.[614] Healthy elderly also have severe symptoms associated with infection, although they are less likely to be hospitalised. They do, however, seek more medical consultations than any other age group with RSV.[361]

In high-risk adults and children with cancer, congenital CPD and immune disorders, RSV infection is of notable importance.[611,612,636] Adult leukaemia or haematopoietic stem cell transplant (HSCT) patients are 80% to 100% at risk of death due to RSV, depending on the category and degree of immune suppression.[611,636-638] Lung transplant patients are predisposed to severe LRTI, chronic transplant rejection, or bronchiolitis obliterans.[639,640] Finally, children with CF are most often hospitalised and have decreased lung function.[30]

8.3.5.1.2.3 Healthy Young Adults

There remain considerable gaps in knowledge of RSV infection in healthy young adults, mostly because cases are left undiagnosed.[32,612] Current literature suggests that RSV reinfection occurs at rates between 5% and 10% annually in healthy adults and is generally associated with mild URTI and cold symptoms.[612,614,641] Frequent reinfection is more characteristic of those with increased exposure to the virus, such as healthcare workers and residents in households with sick children.[611,612,642] Hospitalisations and deaths due to severe disease are rare, although illnesses may nonetheless be consequential in terms of absences from work.[612,643]

8.3.5.1.3 Infection and Immunity

RSV is understood to be a primarily nosocomial infection, whose clinical burden is most recognised in paediatric wards.[633,644,645] It is suggested that clinical manifestations of nosocomial RSV may be different from community-acquired infections, particularly in infants.[633] RSV is implicated in sudden infant death syndrome, but a direct relationship has yet to be established.[611,646] Fever generally accompanies URTI, which later develops into LRTI in approximately 25% to 40% of those aged between six weeks and nine months.[611,626] Bronchiolitis and pneumonia are the most common LRTI manifestations, although, croup is also possible.[611] In infants for whom RSV infection resulted in LRTI, asthma is predominantly observed during childhood. However, it remains to be established whether RSV predisposes infants to asthma later in life, or if asthma increases the likelihood of severe RSV infection in infancy.[361] In neonates, infections are generally either asymptomatic, or present as mild URTI. Bronchiolitis may develop

in severe cases and apnoea is common in premature births.[611,647,648] Severe disease is rare however, and is most often characterised by systemic signs rather than respiratory symptoms, including temperature fluctuations, listlessness and irritability.[611,649] In children, symptoms may be mild URTI that include rhinorrhoea, cough and sneeze, but could also present as wheezing episodes similar to asthma exacerbations[361] that rapidly progress to bronchiolitis or pneumonia, particularly in those with underlying risk factors.[611,650] In the elderly, RSV infection often results in ICU admittance, due to LRTI.[611] This is particularly the case for those in closed settings, with underlying CPD, or immune-challenged.[611,614] The elderly are also more likely to experience nasal congestion and wheezing when infected with RSV, than with any other respiratory virus.[361] In healthy young adults, RSV infection is suggested to be generally symptomatic, and symptoms are often mild and self-limiting. [32,651] Morbidity may be underestimated in this population, however, because common cold symptoms generally do not result in medical consultations, and because there are no over-the-counter therapies specific for the treatment of symptoms, from which the cost of RSV can be extrapolated.[32,651] Adults with increased exposure to children, such as medical personnel, day care staff and household members, are most at risk for RSV infection and symptom development.[611]

Infection, severity of disease and age at primary infection is suggested to be subtype-dependent.[617] In hospitalised infants, RSVA is understood to have a more serious pathology, and result in higher severity indices, than RSVB.[615,617] On the other hand, RSVB is often correlated with infection of those with underlying medical conditions.[615] RSVA is observed to be more common in children < 1 year of age, while RSVB often infect older children.[617,652] Epidemiological correlation with RSV subtype, however, remains debatable.[617]

Host immune responses to RSV infections differ as much as their symptom presentations.[361] Immunity includes induction of neutralising antibodies and cell-mediated virus clearance.[611] Non-maternal serum IgG and secretory IgA protect against reinfection, while cellular immunity is important in symptom resolution.[611] Antibody responses to primary infection is delineated as a cross-reactivity with RSV F protein, which is highly conserved across strains. On the other hand,

response to RSV G protein is decidedly targeted towards specific strains, due to differences in this protein among strains.[612]

In infants, IgA reduces shedding titres and duration by inhibiting virus replication.[653,654] IgA response in primary infections is short-lived, but subsequent reinfection induces more sustained antigen neutralisation.[653] Infants acquire maternal IgG at birth.[655] Figure 6 below shows that high maternal IgG titres are predominant in full-term births; this correlates with primary infection at an older age and milder disease presentation, both of which are protective.[611,655] Note that IgG titres at birth still depends on maternal antibody titres to RSV.[611] The figure also shows that protective immunity by maternal IgG is short-lived; it declines between days 21 and 26 after birth, and is no longer detectable by age eight months.[611] In hospitalised infants, both IgA and IgG are detectable 10 days after symptom onset, and lasts for as long as four weeks.[656]

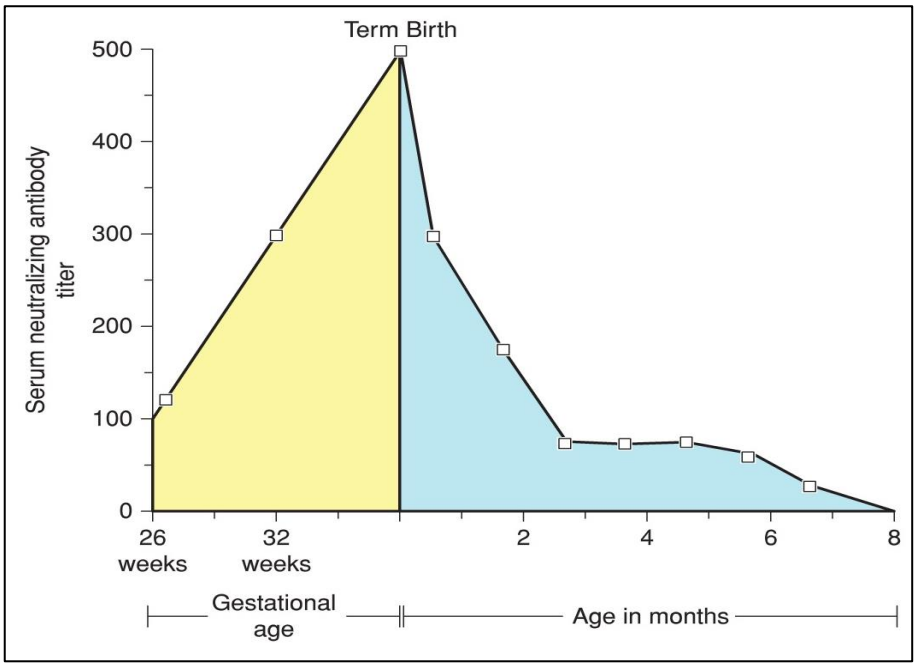

Figure 6 Maternal Serum IgG titres and Duration of Protection in Infants[h]

[h] Taken from Collins and Karron, 2013[611]

Compared to infants < 6 months of age, older individuals have more vigorous and lengthier IgA and IgG response.[611] In healthy young adults, RSV infection induces an eight-fold rise in IgG titres that then decline within a year after infection.[657] In the elderly, the humoral response is more robust than in young adults, suggesting that this age group's increased predisposition to severe RSV infection may not be due to immune senescence, or the age-associated inability to produce antibodies.[611] RSV infection induces partial, but not long-term, antibody activity against RSV F and G proteins. Therefore, reinfection in all populations is frequent, resulting in both symptomatic and asymptomatic illnesses.[612,658] Reinfection in adults is likely due to decreasing titres of both IgG and IgA.[642,659-661] In the elderly, serum antibody titres are likewise inversely correlated to hospitalisation.[611,661,662]

Cell-mediated immunity to RSV is best demonstrated in animal models.[612] In mice, infection induces a Th-1 type CD4 response, producing Th-1 cytokines IFN-γ, and IL-2, along with CD8 CTLs. The response changes to a Th-2 type CD4, producing IL-4, IL-5 and IL-6 without CTL activation, in infections with inactivated whole virus or virus particles.[612] Cellular immunity in humans is not as well studied.[612] CTL response to RSV has not been correlated with protection from infection or recovery from symptoms and the link between cellular induction of cytokines and immunopathogenesis in adults remains to be established.[612,663,664] In infants and children with primary and secondary RSV infection, a vigorous CTL response in peripheral blood is reported.[611,665,666] Severe disease induces both $CD4^+$ and $CD8^+$ T cells response, with the preponderance of CD4.[667,668] In respiratory fluids of infants with severe disease and in lung tissue of fatal RSV cases, however, no notable increases in T-cells have been reported.[611,669,670] CD4 Th-1 IL-4 and Th2 IFN-γ may be correlated to primary RSV infection and reinfection.[611] Serious illnesses characterised by airway plugging due to mucus hypersecretion, wheezing and chronic pulmonary susceptibilities are associated with Th2 bias, much like in asthma.[611] Unlike asthma where prior sensitisation to an allergen is required, however, Th2 activation in RSV is more pronounced in primary infections than in subsequent reinfections.[611] Hence, IFN-γ response in healthy young adults and the elderly is lower than that in children.[361] Comparisons of CD4 and CD8 responses in healthy young adults and the elderly apparently have not been studied.[612]

Immune responses to RSV infection are implicated in severe disease, such as infantile bronchiolitis, where the most serious stage of the disease coincides with peak host inflammatory immune response. This may be due to unbalanced T-cell priming or to ADE.[361]

8.3.5.1.4 Transmission

RSV is extremely contagious.[611] It is pervasive and spreads at speed.[26] Transmission is understood to occur from person-to-person through large droplets produced during coughing and sneezing, and by autoinoculation of the eyes and nose, following contact with contaminated surfaces.[26,645] On the other hand, aerosol transmission has not been qualified as an efficient mode of pathogen transfer, due to RSV being rapidly inactivated in an aerosolised particle.[612,617,671] However, data are limited to observations during nosocomial outbreaks, and where simulations of natural infections involve inoculation of volunteers with different doses of wild-type virus, which may misrepresent real-life situations.[617,671] RSV is also not thought to be able to survive for long periods in the environment because of its labile structure.[26] However, given its communicability, it must persist at least long enough for transmission to occur.[26,617] Extracorporeal RSV survival is suggested to be as long as 12 hours.[672] It has been demonstrated to remain active on nonporous surfaces for approximately six hours, on surgical gloves for as long as two hours, on paper materials for 45 minutes, and on undisturbed skin for 20 minutes.[98,617] However, fomite transmission data are limited to those obtained by experimental contamination of surfaces with virus stocks or secretions containing titres presumed to be comparable to natural infection, rather than actual observations of fomite transmission events.[98,617]

RSV is the predominant cause of nosocomial transmission of respiratory infections.[658] Risk factors for acquired nosocomial infection (NI) include young age, underlying medical conditions, and length of hospital stay during increased RSV admissions.[644,645] Transmission rates in neonatal intensive care units and paediatric wards can be as high as 45%, even with the implementation of infection control strategies.[633,658] Rates in elderly long-term care facilities are similar, at approximately 40%.[617] NI results in longer hospital stays than community

infection and predisposes patients to more serious disease.[633] ICU admissions are as high as 50% in children with nosocomial RSV, compared to 9% of those with community-acquired virus, supportive care is indicated for 2% to 10% of healthcare-associated infections, and mortality is pronounced in nosocomial RSV, whereas it is negligible in the community.[672]

Hospital staffs are a recognised source of NI.[644,672] Healthcare workers are susceptible to both nosocomial- and community-acquired RSV. However, they are rarely identified as disease vectors, because their symptoms tend to be mild, presenting as colds or ILI, such that they are still able to come in to work. [644] Studies during nosocomial outbreaks have demonstrated that approximately 60% of staff are infected when RSV is circulating in the community, and as much as 27% could then infect patients, other staff and people in the community.[633,644] Asymptomatic infection has also been observed in 15% to 20% of HCW, which is often accompanied by high titre shedding.[644] Data on NI are again limited to few studies on healthy young adults in medical care, often during outbreak situations. Medical trainees and newly appointed personnel in paediatric wards are at the most at-risk of symptomatic NI, but are also the least likely to appreciate the implications of their illness,[644] which is one of the reasons why few data are available in this population.

8.3.5.1.5 Prevention and Control

There are no licensed vaccines against RSV. Clinical trials in the 1960s catastrophically resulted in enhanced disease in some and death in others. Subsequent vaccines were safer, but remained unsuccessful in producing sufficient correlates of protection.[611] However, a 2015 WHO committee established a critical need for RSV vaccines and confirmed that there are several pipelines in progress.[673,674] Considerable activity is currently underway in clinical development of vaccines, primarily intended for infants and young children, for whom the burden of RSV disease is greatest. However, there are suggestions that this be extended to other age groups, for which primary care and emergency attendances have the highest impact, structurally and economically.[624,625]

Two predominant candidate RSV vaccines include one containing a purified F protein subunit (PFP-2) and another with cold-adapted, temperature-sensitive, live-attenuated RSV.[612] The latter has undergone Phase I and Phase II trials in infants and young children. Although determined safe and non-pathogenic, it nonetheless remains inconsistently immunogenic.[612,675-677] In adults, PFP-2 and cold-adapted formulations are tested either in combination or sequentially. Again, although safe and well tolerated by healthy young and older adults, immunogenicity is weak.[612,678] Other drugs in production that elicit robust immune responses, however, also enhance disease pathology. This may be due to highly active or irregular T-cell priming or to a magnified inflammatory response due to ADE.[361]

In the absence of a viable vaccine, other methods of prevention and control of RSV infection are in place. These include antivirals, symptom and supportive interventions and behavioural strategies.[611,612] The aerosolised antiviral ribavirin is the only recognised chemotherapeutic for RSV.[612] Its mechanism of activity is not completely understood and its use is hindered by contradicting clinical trial outcomes, difficulties associated with aerosol delivery, development of anaemia in some, and unintended side-effects on those exposed to the aerosol during administration, such as HCWs.[611,679-681] In infants and those at risk of protracted disease, IV monoclonal antibodies (MAb) are administered for prophylaxis against RSV infection. Current studies suggest MAbs reduce shedding, however, clinical outcome has yet to be determined.[611,657,682-684] Hospitalised infants require substantial supportive care. These may include positioning them in a way that helps breathing easier, using automated mucus removal systems, oxygen therapy, or, in severe cases, mechanical ventilation.[611] Antivirals may be used during epidemics for prophylaxis of those with CPD or immunosuppression, who are at high-risk of LRTI, however, they are not indicated for healthy adults with URTI.[612,636,638,685,686]

Management of annual nosocomial RSV outbreaks are attempted with various infection control mechanisms. These are generally behavioural strategies that include patient and HCW cohorting, rapid screening for RSV infection, isolation methods, visitor restrictions, hand washing, and PPE.[617,633,645,672] These methods

are implemented either individually or in combination, but not one single or amalgamation of infection control has been determined to be more effective than another. Although, a combination of rapid diagnostics, proper hand washing and patient cohorting has been shown to have better epidemic management outcomes.[633]

8.3.5.2 Human Metapneumovirus (hMPV)

HMPV belongs to the same *Paramyxoviridae* subfamily as RSV - *Pneumovirinae*. There is a 50% genome sequence homology between hMPV and RSV, and an 80% similarity among hMPV subgroups.[611] Structurally, hMPV is shorter than RSV by almost 2kb.[611,687] Much of the preceding information on RSV also applies to hMPV, including the structure in figure 6. This section, therefore, only highlights areas of importance that are specific to hMPV.

HMPV was first identified in 2001, in paediatric inpatients presenting with symptoms similar to patients with RSV, but that had negative RSV test results.[2,29,361,611,688] Although it is a fairly recently discovered pathogen, seroepidemiology indicates its circulation in humans at least 50 years ago, indicating that it is not a newly emergent virus.[2,29,611,688] Delay in its identification is attributed to its sluggish growth and CPE in cell culture, the need for trypsin in the activation of the F protein, and diagnostic laboratory use of cell lines that do not support hMPV replication.[2,611] Despite its discovery 15 years ago, however, hMPV yet remains to be included in routine respiratory virus surveillance systems. Therefore, much of what is currently known is derived from limited research on the pathogen[2] and, until hMPV data become more widely available through either its inclusion in routine diagnostic testing or its being a more active subject of research, currently available information has to be interpreted with caution.

HMPV is similar to RSV as a clinically relevant pathogen in respiratory disease in paediatrics and the elderly worldwide. However, primary infection occurs slightly later in the first year of life than RSV and severe disease is uncommon. Therefore, its clinical impact is not considered to be as fundamental as that of RSV.[611] Also much like RSV, hMPV has one serotype with two subgroups, hMPVA and hMPVB.

There is considerable cross-reactivity and cross-protection between subgroups.[611,689] However, there is no evidence to suggest an association between subgroup and disease pathology.[611,689]

Primary hMPV infection resulting in hospitalisation is highest at around six months of age, with almost all children being infected by the age of five years.[2,688] Reinfection is common, with recurrence in infants resulting in either URTI or LRTI.[19,611] Yearly reinfection is understood to occur at 1% to 9% in young adults, and range from asymptomatic carriage to severe respiratory infection.[690,691] In the elderly, risk for severe hMPV infection is demonstrated to be highest in those with underlying chronic health conditions.[611,690,692]

8.3.5.3 Human Parainfluenza Viruses (PIV)

8.3.5.3.1 General Information

PIV are also members of the *Paramyxoviridae* family that infect and cause respiratory disease in humans.[611] They belong to the subfamily *Paramyxovirinae*, which is how they differ from both RSV and hMPV.[611] The name parainfluenza derived from their influenza-like pattern of disease, lipid envelope structure, ability to agglutinate blood in culture, and neuraminidase enzyme activity.[693,694] There are four HPIV serotypes, all identified between 1956 and 1960, when cell culture and haemadsorption methods were used to isolate pathogens associated with respiratory tract infection in children.[694] They are: PIV1, PIV2, PIV3 and PIV4. The odd-numbered serotypes belong to the genus *Respirovirus*, while the even-numbered ones belong to the *Rubulavirus* genus.[611] PIV4 is further subtyped into PIV4a and PIV4b, based on antigenic differences in haemagglutination inhibition tests and on susceptibilities to MAbs.[694,695]

PIV1, PIV2 and PIV3 were first detected in infants and children presenting with LRTI, and were directly associated with laryngotracheobronchitis (croup) in children.[694] As a group, PIVs 1 to 3 are considered to be second only to RSV in severe virus-induced respiratory tract illness in infants and children.[694] PIV4, on the other hand, was first identified in children and young adults with milder URTI.

Compared to the other three serotypes, PIV4 causes respiratory diseases only infrequently and with less severe pathologies.[694] However, recent serological indications suggest that these findings could just be due to difficulties in isolating PIV4 in culture, rather than its comparatively diminished ability to cause disease.[694,696-698]

8.3.5.3.2 Epidemiology

PIVs are globally ubiquitous viruses that cause acute respiratory infections in people of any age. However, disease presentation and age at primary infection generally varies with each serotype.[694] Hospitalisation rates due to PIV in children are approximately 38% for PIV1, 12% for PIV2 and 50% for PIV3.[694,699] As a group, PIVs 1 to 3 cause acute respiratory diseases in infants and children that range from mild URTI and pharyngitis to serious LRTI.[694] URTI complications, which include otitis media, are implicated in 30% to 50% of PIV infections in children.[700,701] Infection occurs most often between the ages of three and twelve months, with approximately 60% of children being infected with PIV3 by the time they are two years old.[694,701,702] PIV1 and PIV2 cause disease much later than PIV3 in early childhood, however, most children would have been infected with all three serotypes by the age of five years.[694,703-705] Croup is also most often associated with PIV1 and PIV2 infections between the ages of one and two years, and children remain susceptible to this LRTI until the age of six years.[694,700,706] Approximately 65% of croup cases are due to PIV1 infection alone.[707] On the other hand, severe disease due to PIV3 infections result in pneumonia and bronchiolitis in the first six months of life, but rarely occur this early with PIV1 and PIV2.[694,706,708,709] In comparison, PIV4 is less symptomatic and causes a broad spectrum of illness in both children and adults.[694,696-698] Its incidence in paediatric populations is similar to that of PIV2, at approximately 10% annually.[694,698,701,710]

PIV is primarily an infection in young children. However, adults may also be susceptible to disease, which is generally characterised by mild URTI and common cold symptoms.[434,701] Adults hospitalised with LRTI are usually those infected with PIV1 and PIV3, but not PIV2; the greatest severity is due to PIV3.[701,711] LRTI in adults may be due to community-acquired pneumonia resulting from PIV

infections.[711] Complications associated with LRTI include wheezing and pulmonary murmurs caused by PIV1,[711] while PIV3 is associated with adult croup and in 27% of adult hospitalisations for lobar pneumonia.[701,712] Figure 7 below illustrates the proportions and age distributions of each HPIV serotype, from laboratory data collected by Laurichesse *et al*. (1999) in England and Wales from 1975 to 1997.[701,702]

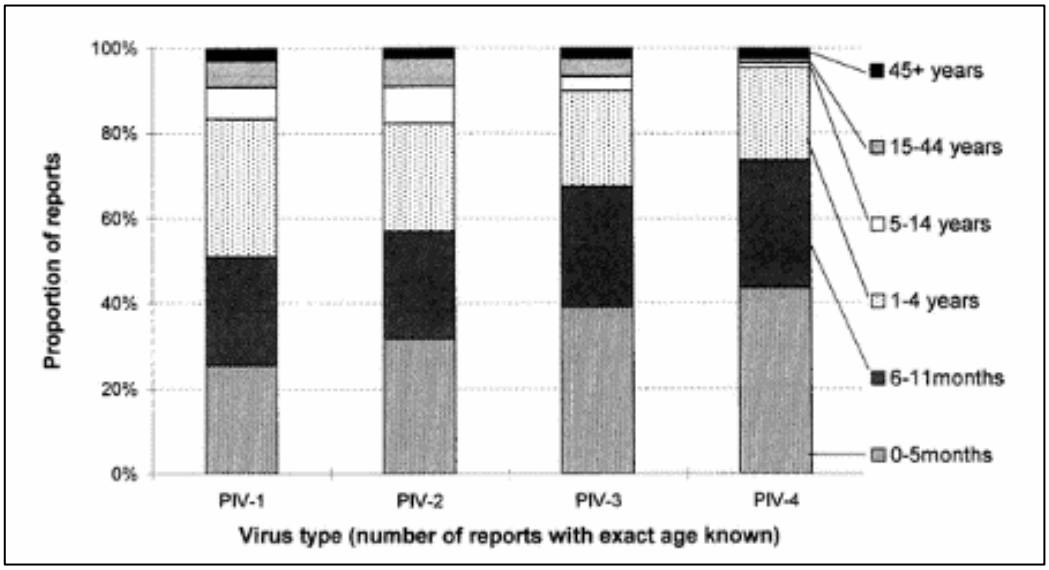

Figure 7 Age Distribution of PIV Reported in England and Wales, 1975-1997[i]

Annual epidemics in temperate regions, including England and Wales, may account for the higher incidence of PIV3 infections in the overall population than PIV1 and PIV2, which have biennial patterns of outbreaks. However, recent evidence suggests that PIV2 may also now have annual epidemic patterns, and that PIV1 may have peak incidence at low levels around February each year.[694,701,702,711] Also in temperate regions, PIV1 outbreaks are associated with increased consultations for croup, for a period of 13 to 15 weeks between September and December, in odd-numbered years.[701,707,713-715] Its biennial pattern of epidemic is most pronounced in children <5 years old, while recent annual peaks are mostly seen in older children.[701,711] PIV2 may co-circulate with PIV1, although it is most active for a period of up to 18 weeks between October and December.[701,713] PIV3 infections

[i] Taken from Laurichesse et al., 1999[702]

persist for longer periods than PIV1 and PIV2, generally occurring between April and June each year. However, in the years where PIV1 outbreaks do not occur, PIV3 becomes more prevalent, resulting in a protracted activity in spring, followed by an additional epidemic in autumn.[701,713] PIV4 epidemic patterns are not as well delineated.[701,713] Approximately 75% of PIV4 isolates identified are subtype B, however, serological tests show a higher rate of antibody development to subtype A, at almost 95%.[701]

PIV reinfection commonly occurs in older children and adults. Reinfection may be promoted by virus ability to survive for long periods in the human population without undergoing appreciable antigenic changes, which is a fundamental feature of PIVs.[694] Disease following reinfection is generally characterised by URTI, except in rare occasions, when LRTI may result in hospital admissions.[694,711] Death in healthy individuals due to PIV are also understood to be rare, although it has not been studied well.[694]

8.3.5.3.3 Infection and Immunity

Primary infection with PIV generally results in symptom development. These include coryza, sore throat, cough and hoarseness, often accompanied by temperatures >37.8˚C that last for up to three days.[704,705] Severe symptoms associated with LRTI in children may result in respiratory distress that is generally only associated with PIV in adults.[716]Croup is the signature clinical syndrome of PIV LRTI infections in children.[701]

In patients with primary or acquired immune deficiencies, PIV infection may spread to organs and tissues. This results in severe and prolonged diseases that increase rates of morbidity and mortality in this population.[694,701,717] PIV3 is the most frequently identified serotype in immune-challenged populations.[718] On rare occasions, PIV3 can also infect the CNS, resulting in meningitis, and syndromes of Guillan-Barre and Reye's disease in children and adults.[719-723] Febrile convulsions may also arise in immunosuppressed children, usually due to infection with PIV4b.[701]

PIV infections induce effective, robust local and systemic host immune responses.[694] Antibodies specific to PIV surface glycoproteins are the most capable

of neutralising these viruses.[724] In infants, the titres of passively acquired maternal IgG are generally inversely proportional to the likelihood of PIV3 infection in the first four months of life.[703] Serum neutralising antibodies may also be protective for some infants and children during PIV outbreaks in closed settings.[703] In adults, secretory IgA response to PIV1 and PIV2 are associated with protection from disease and URTI.[725,726] Serum antibody levels generally have long durations of persistence,[694] while IgA and cellular $CD8^+$ CTL immunity are short-lived, accounting for the frequency of reinfection throughout life.[694] CTL response is cross-reactive among all serotypes, however.[694] Reinfection may be further promoted by PIV interference in host IFN activation pathways.[694,727] Persistence in healthy individuals is rare, although it may be different in the immunocompromised.[694]

It is suggested that a robust host immune response to PIV infection may be the cause of a severe pathology. In particular, PIV-specific IgE antibody production is induced earlier and at higher concentrations in infants and children with croup, than in those presenting with URTI. Consequently, children with croup shed more histamine through the nasopharynx than those with milder symptoms.[694,728] Similarly, vigorous IgE and cell-mediated immune responses are customarily observed in infants with PIV bronchiolitis.[728] However, it remains to be determined whether host IgE and cellular immunity responses in LRTI-associated PIV infections in children are indeed unusually aggressive, or merely proportional to greater virus pathogenicity.[694]

8.3.5.3.4 Transmission

PIV transmission has been studied most in children and is understood to occur primarily through direct person-to-person contact or by large droplet infection of the nose and throat.[694,701] The incubation period range from two to eight days, after which, LRTI may develop [701,714,729] PIVs have not been demonstrated to survive long in the environment. However, it is observed that infection spreads rapidly within families and in semi-closed settings and healthcare facilities.[694] Infection in families with children occurs at a high frequency.[703] Preschool children are the common sources of household transmission, which occurs at approximate

rates of 59% for those between two and five years old.[694] Nosocomial transmission is of particular concern in paediatric wards and for immunocompromised patients.[694,718,730,731] Outbreaks are generally due to sporadic transmission of multiple PIVs either in hospital or in the community, and HCWs are often critical to the spread.[694,701,730] Incubation periods in hospitals are shorter than in the community, with shedding occurring two to four days after infection.[708] Shedding of PIV3 generally lasts for a period of up to 10 days during primary infection but shorter in reinfections.[708] Prolonged shedding of up to four weeks may also occur in infants and young children, and in adults with underlying chronic respiratory conditions.[46,732] However, it is not known how long it takes for the virus to spread.[708] PIV3 is the most efficiently transmitted serotype from person-to-person, particularly in semi-closed daycares.[694] Individuals that have had prior PIV3 infections may not become ill but may nonetheless be contagious.[694,708]

8.3.5.3.5 Prevention and Control

There are no licensed PIV vaccines for humans. Initial candidate vaccines were not satisfactorily immunogenic and failed to provide correlates of protection against disease.[694,701] Infants <6 months of age are at highest risk of PIV3 infections, but are also the least likely to benefit from live, attenuated vaccines, because of either immunologic immaturity or suppressed humoral immunity due to the presence of maternal serum IgG.[733,734] The use of killed PIV virus is not an active area in vaccine development.[694]

Similarly, development of therapeutic strategies for the prevention and control of PIV infections is limited, because the clinical burden of the virus is primarily in very young children with croup.[701,714] Hence, treatment is focused instead on supportive care and symptom relief using corticosteroids and epinephrine.[694,701]

Although PIV is considered a disease of the young, the preceding information suggests that they may also be relevant in adult respiratory disease. Therefore, more epidemiological data are needed in PIV infections in adults.

8.4 Addressing Knowledge Gaps using TraVerse

The literature review above illustrates that, although there is vast knowledge on viruses associated with the common cold, there remain gaps in our general understanding. In particular, there are disparities in associating virus with primary mechanisms of human-to-human transmission, and in establishing the epidemiology of the common cold in healthy young adults. In aiming to address these disparities, this study used the TraVerse method in combination with state-of-the art molecular diagnostic methods, the results of which are discussed in later chapters. Aside from providing the first working model in ascertaining the mechanisms of natural respiratory transmission of acute infections, and contributing data on disease burden in young adults, it was anticipated that this study would augment knowledge specific to individual viruses. These include 1) disease severities in Flu, PIV, RSV and RV infections, relative to subtype and viral load, 2) rates of transmission when prolonged and continuous human contact is not involved in RV, 3) data on acute infections with EV and RSV, to supplement household studies and nosocomial findings, respectively, 4) supportive data for either aerosol or fomite transmission of flu 5) information on endemic and asymptomatic infections with HCoV, 6) basis to encourage hMPV inclusion in outine surveillance, and 7) the incidence of asymptomatic infection with RSV.

9 Materials and Methods

9.1 Setting

The Children's Hospital based at the Leicester Royal Infirmary (LRI) is the only inpatient paediatric clinic in Leicestershire, UK. It provides care to a population of approximately 24,000 children aged from birth to four years, and 45,000 aged from five to fifteen years.[735,736] The LRI offers Accident and Emergency (A&E) services, with the Children's Emergency Department (ED) separate from the adult facility.[737] Following primary care or ED assessment, children are admitted for specialised consultation at the Children's Assessment Unit (CAU). Subsequently, they are either transferred to the acute paediatric wards, or discharged, with rapid access to outpatient clinics for up to 48 hours.

The CAU has two 'see and treat' rooms where patients not assigned beds or cots are assessed, and each of the wards has a 'treatment' room for clinical procedures. These rooms were the controlled settings where the child-healthy volunteer interactions of this study were closely observed.

9.2 Volunteers

9.2.1 Children

Children acted as potential infectious sources for respiratory virus transmission, and hence are known as 'source' hereafter. Sources were patients aged from birth to six years, who presented to either the ED or CAU between November 2012 and May 2015, and who satisfied study-specific inclusion/ exclusion criteria (Appendices A1 and A2). Briefly, children were recruited if they were born after 32 weeks' gestation, presented within 216 hours (9 days) of onset of fever (≥37.8˚C), respiratory tract infection, or gastrointestinal conditions, and had no cardiac or metabolic abnormalities.

9.2.2 Healthy Young Adults – Hosts

Healthy young adult volunteers acted as virus hosts and are known as 'host' hereafter. Hosts were non-asthmatic University of Leicester (UoL) medical students aged 18 to 35 years that met study-specific inclusion and exclusion criteria (Appendices A3 and A4). Included in these criteria were the absence of respiratory symptoms for up to 14 days prior to interaction (day 0), and oral temperature <37.4°C on day 0. Pregnant females were excluded from participation.

Hosts were screened for participation during study information sessions provided by the researcher and other members of the study team, generally at the start of school terms in September and January each year. At these sessions, hosts were informed of the nature of the study and provided detailed, standard information sheets. At the end of each session, written consent was taken from those that satisfied study criteria, using standard consent forms. Consenting was done according to the tenets of the Declaration of Helsinki[738] and Good Clinical Practice in Research (GCP),[739] for which the researcher received training and certification.

Consented hosts provided contact details and fortnightly schedules of availability via email, so that only those at hand were called upon once a source has been recruited. Schedules were managed using Anytime® Organizer Deluxe 15 software (Individual Software Inc., USA). Figure 8 shows the workflow for screening and recruiting hosts.

9.2.3 Healthy Young Adults – References

In the final year of the study, nose and throat swabs were taken from adults who have not participated within a 28-day period, for reference purposes. Data from these references were used to ascertain whether any viruses were circulating in the community at the time of sampling that may result in hosts testing positive during the follow-up period. If reference hosts test positive for viruses that were circulating at high levels in the community (based on public health data), then it would bring up the possibility that virus-positive hosts who interacted with

sources during that time could have acquired the virus from the community and not from source. The number of reference subjects that participated equated to approximately 10% of the number of interactions.

9.3 TraVerse Method –Design and Conduct

9.3.1 Day 0 - Immediately Prior to Interaction

9.3.1.1 Source Recruitment

Once a child had been admitted into ED or CAU and assessed by senior medical personnel to fit the study profile for a potential virus source, the researcher approached primary guardians to consent to the child's participation in the study. Detailed study information sheets were provided and guardians were given at least one hour to make an informed decision in providing consent. Guardian consent was taken under the same conditions as in hosts, using standardised forms. Consent was taken within 234 hours of onset of illness, so that interaction with healthy adult volunteers was completed within 240 hours (10 days).

Following consent, demographic information and clinical history were collected from patients' notes onto standard case report forms (CRFs).

9.3.1.2 Host Clinical Assessments

Hosts available at the time guardian consent was obtained were contacted by text message and asked to arrive in clinic within 30 minutes. Because hosts were paid to participate, the researcher established fairness by contacting them in the order in which they submitted their schedules of availability. Hosts were given 30 minutes to respond, after which, the next person on the list was contacted.

Upon arrival at clinic, hosts were re-consented into the study, to re-affirm that the risks involved were clearly understood and accepted, and to ensure that inclusion/ exclusion criteria for day 0 were met (Appendices A3 and A4); specifically, that respiratory symptoms were absent for 14 consecutive days.

Upon consent, host oral temperature was taken by the researcher, using a medical-grade, digital thermometer (DigHealth, China; catalogue no. YGH-055). Women were asked to take a pregnancy test using a rapid, mid-urine stream kit that provided results in three minutes (Alere, USA; catalogue no. UPK009A). Clinical information on respiratory symptoms, oral temperature and pregnancy tests were recorded on standard CRFs and in standard diary cards (Table 3). These were the baseline (day 0) clinical conditions of the host.

Interaction with source proceeded only once it was determined that the host had an oral temperature <37.4°C and negative respiratory symptoms and pregnancy test. The researcher continued by collecting baseline blood samples and nose and throat swabs (see section 9.4 on collection of blood and swabs), before escorting the host to the controlled setting where interaction was to occur.

9.3.1.3 Preparation of Controlled Setting

Upon arrival at the interaction room, the host proceeded to hand washing with soap and water at the room sink, while the researcher brought in age-appropriate toys and promptly swabbed a selection. The host was informed of which toys were swabbed, to ensure that both source and host handled them at least once during the interaction. The researcher also introduced a portable bioaerosol sampler containing an air filter into the room (see section 9.4 on collection of toy and aerosol samples), which stayed in the 'off' position until interaction commenced.

9.3.2 Day 0 – During Interaction

Accompanied by a guardian, the source was brought into the controlled setting to interact with the host for a maximum of 30 minutes. The entire event was supervised and facilitated by the researcher, while donned in PPE that constituted of apron, face mask and gloves. Source clinical samples that consisted of nose, throat and hand swabs were collected at this time. The bioaerosol sampler was switched to the 'on' position.

During the 30-minute interaction, both host and source were kept within three feet of and facing each other at all times, while they carried out three 10-minute cycles of playing with age-appropriate toys for four minutes, clinical assessment simulation for five minutes, and host rubbing of own face for one minute (Figure 9). On occasions that the source did not participate well, the interaction was limited to five minutes – four minutes for playing and one minute for host face rubbing.

9.3.3 Day 0 - Immediately After Interaction

At the end of interaction, the source was sent back to the patient bed; this marked the end of source participation. The bioaerosol sampler was switched off, and the filter inside was transferred into virus transport medium (VTM). Clinical samples were then taken from the host, consisting of hand, face and clothing swabs. Toys that were swabbed prior to interaction were again swabbed. All clinical and environmental samples were then delivered to the laboratory for immediate processing.

The host was handed the diary card on which the researcher recorded baseline clinical conditions prior to interaction. The host was also provided with a medical-grade digital, oral thermometer and given instructions on accurate temperature recording.

9.3.4 Days 1 to 10 Follow-up

Host follow-up required three-times-daily completion of the diary card with symptom and temperature logs at 0900, 1500, and 2100 (all ±two hours), beginning at the first opportunity after the interaction on day 0, through to the subsequent 10 days.

The diary card included a list of 21 signs and symptoms (Table 3) generally associated with ILI.[740] Hosts were required to score each of the 21 listed as '0', '1', '2', '3', or '4', respectively for none, mild with no limitations to normal activity,

moderate and some limitation to normal activity, severe without needing medical attention, and incapacitating and needing medical consultation. If symptoms were present, hosts were instructed to indicate which of three actions were taken, if any: stayed home, sought medical care, or self-medicated with antipyretics or analgesics, including the dose and duration of use. Hosts were further instructed to contact the attending research clinician, should they spike temperatures ≥37.8˚C, so that they may be referred to a GP for antiviral administration.

Follow-up also required that the host returned to the research clinic for a maximum of four follow-up nose and throat swab sampling, whether symptomatic or not. Follow-up swabs were taken once on day 1, days 3 to 5, days 6 to 7 and days 8 to 10.

Hosts were eligible to participate up to three times. However, a 12-week period of symptom recovery and virus clearance was imposed after every interaction, unless prior interaction resulted in negative PCR tests or symptom development; then only a 28-day period of rest was imposed. Each interaction with source was counted separately.

9.4 Clinical and Laboratory Methods

9.4.1 De-Identification of Samples

For the purposes of blinding the researcher to sample origin and de-identifying sources and hosts, all samples were assigned consecutive numbers and labelled with the volunteers' initials, the interaction number and the date of collection.

9.4.2 Equipment Sterilization

To ensure that carry-over of viruses from previous interactions did not occur, devices were sterilised after each use and stored until next needed. Toys were disinfected by soaking in 1% broad spectrum biocidal (Virkon, USA; catalogue no. CL/454), in accordance with the UHL/ NHS 'Infection Prevention and Control

Guidelines for Selecting, Maintaining and Cleaning Toys' (INsite Document Number 37741). Toys were then towel-dried prior to storage. In compliance with the manufacturer's guidelines for device care and storage, bioaerosol samplers were wiped with single-use 70% isopropyl alcohol disinfection wipes (Clinisupplies, England; catalogue no. NW/NWSWAB500/B) and then air-dried prior to storage in a case provided by the manufacturer.

9.4.3 Blood Collection

The researcher received training and certification to perform phlebotomy. Venous bloods (up to 9 mL) were collected in an S-monovette polypropylene clotting activator tube (Sarstedt Germany; catalogue no. 02.1063) with an S-monovette 21 G x 1 ½ needle (Sarstedt, Germany; catalogue no. 85.1162). Upon collection, blood tubes were centrifuged at 3000 rpm for five minutes. Sera were then collected from clotted blood; equal aliquots were transferred into three labelled, 1.8 mL-volume, sterile, cryogenic vials (Thermo Fisher Scientific, England; catalogue no. 11311665), and then stored at -20˚C for future serological analysis.

9.4.4 Nose and Throat Sample Collection

A flexible Hydraflock® swab (MWE, England; product no. MW819125) was inserted first into one nostril and then the other and placed in a tube containing 1 mL of VTM (MWE, England; product no. MW951S). A foam swab that was provided with the VTM was then used to abrade the tonsils and pharynx and then placed in the same tube as the nose swab. Both swab tips were broken off at the perforation line before the tube was capped and labelled, for immediate transport to the laboratory. Upon arrival, VTM tubes were agitated with a vortex mixer for one minute before they were opened. Swabs were then aseptically squeezed against the side of the tube before being discarded in biological waste bins. Equal aliquots of VTM were transferred into two labelled, 1.8 mL-volume, sterile, cryogenic vials, before storage at -80˚C for batch PCR analysis.

9.4.5 Toy Sample Collection

Reusable, age-appropriate toys were procured following the advice of LRI play specialists, and stored in separate age-labelled containers for easy sorting. Toys were CE-marked and compliant with EU 'Safety Standard EU EN71' and 'Toy Safety Directive 2009/48/EC.' Using a foam swab that was provided with VTM, the entire surface of toys was swabbed immediately before and soon after interaction. Pre- and post-interaction swabs were then placed in separate labelled VTM tubes and immediately transported to the laboratory for batch PCR analysis.

9.4.6 Bioaerosol Collection

A 25mm, portable button aerosol sampler (SKC, USA; catalogue no. 225-360) was used in combination with a 25mm-gelatin filter (SKC, USA; catalogue no. 225-9551), for the collection of inhalable viruses at a maximum flow rate of 4L/min., in accordance with ACGIH/ ISO criteria.[741] The filter was aseptically inserted into the sampler immediately prior to each interaction. The assembled device was then introduced into the setting where the interaction occurred; it was set to the 'on' position when both source and host were present, and turned off soon after interaction. The filter was then aseptically removed from the sampler, transferred into a labelled VTM tube and immediately transported to the laboratory for batch PCR analysis.

9.4.7 Real-time RT-PCR

9.4.7.1 Nucleic Acid Extraction

RNA and DNA from swab samples were extracted and purified using the automated QiaSymphony SP (Qiagen, Germany) nucleic acid extractor, with a DSP/ Virus Pathogen Mini kit (Qiagen, Germany; catalogue no. 937036). The kit contained all buffer reagents in pre-filled cartridges and consumables for up to 96 samples. QiaSymphony uses a magnetic bead technology to isolate and purify

nucleic acid from clinical samples. Up to 24 samples can be processed each time; the procedure was completed in approximately 1-¼ hours.

Prior to automated extraction, off-board lysis was performed to inactivate viruses and lyse human cells; a 200µL sample in VTM was pre-treated with an equal volume of buffer ATL (Qiagen, Germany; catalogue no. 939011) for 10 minutes. Lysed samples were then transferred onto the extractor, which was pre-programmed to automatically set into motion the process of 'bind, wash and elute.'[742] The extractor was also pre-programmed to spike samples with 2µL of 1 nM MS2 bacteriophage carrier RNA (Roche Applied Science; catalogue no. 10165948001) as an internal control; this was an indicator of effective nucleic acid extraction and any RT-PCR inhibition. The extracted and purified virus RNA and DNA (eluate) from each sample was then released into 60µL of elution buffer for subsequent RT-PCR amplification.

9.4.7.2 Qualitative and Quantitative Real-time RT-PCR

9.4.7.2.1 Assays and Panels

Real-time, qualitative and quantitative RT-PCR were performed using assay panels developed at the Clinical Microbiology Department of the Health Protection Agency (HPA) - Addenbrooke's Hospital, Cambridge, UK and the at the HPA-Colindale as previously described.[743,744] There were five multiplex, subtyping assay panels used in this study. Panel 1 was for subtyping of Flu H1, H3, and B and for the detection of MS2 internal control; Panel 2 subtyped RSVA and RSVB; Panel 3 was for EV and RV; Panel 4 identified HCoV group 1 (NL63 and 229E), group 2 (OC43 and HKU1), and SARS; and Panel 5 subtyped PIVs 1,2,3 and 4. Simplex PCR assays were used to identify HAdV, hMPV and FluA(pandemic H1).

Quantitative RT-PCR was used to determine viral load in copies / mL for EV, FluA (pandemic H1), FluA(H3), FluB, RSVA, RSVB, and RV. Amplirun whole genome plasmids for each of these viruses were purchased directly from the manufacturer (Vircell, Spain; catalogue nos. MBC082, MBC029, MBC030, MBC041, MBC083, MBC091 and MBC019, respectively). Each 50µL volume was serially diluted with PCR-grade water provided with the plasmids, to contain $\log_{10}$ viral loads between

500 copies / mL and 5,000,000 copies/ mL. Dilutions were used to generate a standard curve for absolute quantitation. A 1:2 dilution of the standards containing 50,000 virus copies /mL was used as external positive controls for the assays.

Qualitative RT-PCR was used to detect the presence of HAdV, HCoV (including SARS), hMPV, and PIV. The SARS positive control was provided by HPA-Cambridge, while positive controls for the other assays were stock samples used in external quality assurance proficiency test panels by the Clinical Virology Department of the LRI, which were provided to the researcher free-of-charge. An additional qualitative RT-PCR for the detection of human RNAseP gene previously described,[745] was used to verify that nose and throat swab samples were properly collected and that samples that tested negative were true negatives, and not resulting from poor sampling.

9.4.7.2.2 PCR Master Mixes

Primers and probes for each assay were pre-mixed to streamline laboratory procedures and to ensure the uniformity of master mixes in each run. Primer-probe mixes were made up for 500 reactions at a time, which were then separated into aliquots for 10 and 20 reactions, prior to freezing at -20°C. Aliquots were used to ensure that freeze-thaw of mixes only occurred once. All mixes were made up using PCR-grade water (Severn Biotech Ltd., England; catalogue no. 20-9000-01) and all aliquots were stored in 2 mL-volume RNAse and DNAse-free cryogenic vials (Nalgene, USA; catalogue no. 5011-0020).

The sequences, concentrations and volumes of primers and probes for each assay are listed in tables 4 to 12. Primer-probe mixes were used in making up PCR master mixes with the SuperScript III (SS-III) One-Step RT-PCR System (Invitrogen, USA; catalogue no. 1257-4026). PCR master mix recipes are shown in table 13.

9.4.7.2.3 PCR Set-up and Analysis

One PCR amplification reaction contained a 25μL-volume of master mix (20μL) and extracted nucleic acid (5μL), in a 0.1 mL strip tube with cap (Qiagen, Germany; catalogue no. 981103). Amplification was carried out in a 72-well rotor (Qiagen, Germany; catalogue no. 9018903) on a Rotor-Gene 3000 thermal cycler (Qiagen, Germany), with Rotor-Gene Q software (version 6) for data analysis. Thermocycling conditions were the same for all assays and consisted of 30 min at 50°C for reverse transcription, 2 min at 95°C for strand denaturation and SS-III enzyme activation, and 45 cycles of 15 s at 95°C (not acquiring) and 1 min at 60°C (acquiring on all channels). Each run included 24 samples, one positive control, one no-template control and one negative control; quantitative runs also included at least 4 standards.

A positive RT-PCR determination was defined as the detection of cycle threshold (Ct) value ≤45, with all positive and negative controls yielding expected values. Viral load concentrations greater than the highest standard dilution were reported as >5 million copies/ mL. Concentrations lower than the lowest detectable standard were reported as <5,000 copies/ mL for all viruses except FluA(H3), which was reported as <500 copies/mL.

9.4.8 DNA Sequencing and Analysis

To establish if transmission occurred where hosts were determined to have shed the same virus as their sources during the follow-up period, but also prior to interaction on day 0, source and host nose-throat samples were subcontracted for commercial sequencing (Eurofins Genomics, England) so that virus genotype may be determined. Primers used in RT-PCR amplification were the same ones used in sequencing. A nucleotide Basic Local Alignment Search Tool (BLAST) in GenBank was then used to ascertain the proportional similarity between sequences of host genotype and that of the corresponding source.

Phylogenetic analyses of sequenced samples were attempted using the neighbour-joining method within MEGA 7[746,747] and Lasergene softwares (DNAStar, USA), to construct dendrograms that elucidate the clustering of source and host virus types, relative to reference virus strains in GenBank.

9.5 Outcome and Measure Definitions

9.5.1 ILI Determination

Host daily symptom scores were determined according to previous studies.[13,24] In summary, symptom scores for each of three daily readings were combined and the average taken, to get daily mean scores. Daily mean scores <6 were associated with the absence of ILI, a score of 6 was mild ILI, scores between 7 and 11 were moderate, and scores ≥12 were severe. Influenza-like illness was further confirmed by symptom durations of ≥3 days during follow-up.

9.5.2 Transmission Determination

Source-to-host virus transmission was considered to have transpired when at least one of the following occurred: 1) virus shed by the host on follow-up days one to ten was the same as that identified from source nose, throat or hand swabs, 2) host hands or face tested positive for the same virus as the source, 3) genetic analysis of source and host virus confirmed similar sequence identities (≥90% identical), or 4) the host developed ILI with a minimum symptom score of 6, and that lasted at least 3 days during follow-up.

In terms of mechanisms of virus transfer, transmission by large droplet occurred when source virus shedding resulted in host disease (shedding or ILI). If large droplet transmission was accompanied by a positive post-interaction aerosol test, then aerosol transmission transpired; if source and host hands were positive for the same virus, then hand-to-hand transmission transpired; if host hands and face had the same virus, then self-inoculation happened; and if toys or host clothing had the same virus as the source, then fomite transmission occurred.

For samples that were sequenced, transmission was determined to have occurred when two criteria were met: 1) the virus strain that was shed by the host during follow-up was not the same as that shed prior to interaction on day 0, and 2) the host strain genotype during follow-up was determined by BLAST analysis to be ≥90% similar to the genotype of virus shed by the corresponding source.

9.6 Measurement of Temperature and Relative Humidity

Daily temperature and relative humidity measurements were taken at the controlled settings at CAU, using calibrated digital thermometer-hygrometers (FunIn, China; catalogue no. ATH 802). Readings were taken once daily before recruitment of sources commenced, and each time during the interactions.

9.7 Data Analysis

Clinical and laboratory data were inscribed in standard CRFs and then entered into a Microsoft® Access (version 2010) database for statistical analyses. Descriptive statistics was used to derive the means ± standard deviation (SD) for normally distributed variables and the medians and interquartile range (IQR) for non-normal distributions. Comparative statistics was performed using chi-square analysis, Student's t-test for normal distributions, and the Mann-Whitney U-test for non-normal parameters. Epidemiological data were calculated using open source statistical software (www.openepi.com).[748]

Alpha level of 0.05 was used for all two-tailed statistical tests, with p values >0.05 considered not statistically significant, and p values <0.001 considered extremely statistically significant.

9.8 Ethics and Audits

This study received favourable approval from the Derby National Research Ethics Service, Derbyshire, UK (REC reference number 12/EM/0341). It was evaluated by an industry-recognised external auditor (Truemall Hall Associates, UK), and was

found to have been very well executed and to have met standards in Ethics and Good Clinical Practice in Research.

10 Results

10.1 Recruitment and Follow-up

10.1.1 Paediatric Sources

Between November 2012 and May 2015, a total of 154 paediatric inpatients were recruited as virus sources. Of these, 43 (28%) were unable to interact with healthy young adults. Rapid patient discharge to free up hospital bed space was the foremost reason for missed interactions. Figure 10 is a flowchart of paediatric source recruitment that specifies the circumstances that resulted in missed interactions and the number of occasions that each event prevented sources from interacting with hosts.

The onset of paediatric illness at presentation ranged from a few hours to four days and consent into the study occurred between one and twenty-four hours after admission. CAU was the location where 98% (109/111) of sources were consented and where interaction subsequently occurred.

10.1.2 Healthy Young Adult Hosts

A total of 191 healthy young adults were screened as virus hosts. All but three (98%) satisfied study-specific criteria and provided consent. Hosts made a total of 552 visits to the research clinic on day 0 and during follow-up. The maximum number of four follow-up swabs was collected from 98% (109/111). All hosts returned completed diary cards and none was lost to follow-up. Figure 11 is a flowchart illustrating the number of adults that were screened and consented into the study.

10.2 Demographics

10.2.1 Paediatric Sources

Sources that participated had a male-to-female ratio of three to two, were aged from birth to 6 years, primarily of Caucasian race, and mostly unvaccinated for influenza. Table 14 characterises source demographics by age, gender, ethnicity and flu vaccine status.

10.2.2 Healthy Young Adult Hosts

Hosts that were consented into the study were equally distributed by gender, aged from 18 to 34 years, primarily Caucasian, and largely unvaccinated for influenza. Host demographics characterised by age, gender, ethnicity and flu vaccine status are also presented in table 14.

10.3 Interaction

10.3.1 Number of Interactions

There were 111 interactions that occurred between paediatric sources and adult hosts. Interactions commenced as soon as twenty min. and up to three hrs. following source guardian consent, with a median start time of one hr. (IQR 0.8 – 1.4). Hourly distributions of interactions that occurred following parental consent are shown in figure 12.

10.3.2 Duration of Interactions

Source-host interactions lasted from five min. to thirty min., with a median length of 25 min. (IQR 20 – 30). Forty-five (41%) interactions lasted the full 30 min. Figure 13 shows the duration of interactions in five-minute intervals, and the proportion of the 111 interactions that occurred during each interval.

10.3.3 Healthy Young Adult Hosts That Interacted

Of the 188 consented adults, 80 interacted with 111 sources. Of these, 24 (30%) participated more than once. The remaining consented adults were not able to act

as hosts, primarily because their academic schedules precluded participation. The distribution of consented adults that participated in the interactions is included in the flowchart in figure 11.

10.4 Samples Collected

A total of 834 nose-throat, hand, face, clothing, toy, and aerosol swabs were collected before and after the 111 source-host interactions. An additional 11 nose-throat swabs were collected from adult references during the last year of study, and 111 host sera were stored for future analysis. Table15 enumerates all samples collected from paediatric sources, adult hosts, adult references, fomites and the environment on day 0 and during follow-up days.

10.5 Laboratory Results

10.5.1 RT-PCR

A total of 845 swab samples were processed for batch RT-PCR analysis. All samples were determined to be satisfactory in that, all tested positive for the presence of the MS2 internal control and provided unequivocal RT-PCR results. Furthermore, human RNAseP gene was detected in all nose-throat samples from sources and hosts. RT-PCR results for all swab samples collected are described below.

10.5.1.1 Paediatric Sources

Overall, 103 out of 111(93%) sources shed virus through their nose and throat. Of these, 57 (55%, 95% CI: ± 0.10) shed one virus, 36 (35%, 95% CI: ±0.09) shed two viruses, and 10 (9.7%, 95% CI: ±0.05) shed three viruses. RV, EV and RSVB were the three most commonly detected viruses, with respective detection rates of 65% (95% CI: ±0.09), 25% (95% CI: ±0.08) and 20% (95% CI: ±0.08). A complete list of viruses and their respective rates of detection in paediatric source nose-throat samples are provided in table 16.

Meanwhile, 34 out of 74 (46%) source hand swabs tested positive. Twenty-two (65%, 95% CI: ±0.09) had one virus, eleven (32%, 95% CI: ±0.09) had two viruses, and one (3%, 95% CI: ±0.03) had three viruses. RV, RSVB and HAdV were the most often identified viruses on source hands, with respective detection rates of 47% (95% CI: ±0.10), 32% (95% CI: ±0.10, and 21% (95% CI: ±0.08). A complete list of viruses and the proportions detected in source hand samples are also provided in table 16. The median age of sources with virus on their hands was 18 months (IQR 14-25).

Six hand-positive sources were not shedding virus through the nose and throat at the time of sample collection. Of these, three had HAdV, two had RV, and one had RSVB.

10.5.1.2 Healthy Young Adult Hosts

Twenty-four of the 111 hosts (22%) tested negative prior to interaction on day 0, and then shed virus through the nose and throat during the follow-up period. Twenty-one (88%; 95% CI: ±0.07) shed one virus, and three (13%; 95% CI: ±0.07) shed two viruses. The viruses shed by hosts during the follow-up period and the corresponding rates of shedding are listed in table 16.

Five out of 19 (26%) host hand swabs were positive for one virus following interaction with sources. Four out of 19 (21%) host faces also tested positive post-interaction. Three (75%; 95% CI: ±0.09) had one virus and one (25%; 95% CI: ±0.09) had two viruses. Viruses identified from host hands and face, along with their rates of detection, are also listed in table 16.

10.5.1.3 Healthy Young Adult References

Three out of 11 (27%) reference adults were determined to have had mono-infections of EV, FluB and RV at presentation to the research clinic. There were no reported outbreaks of any of the respective viruses in the community at the time reference samples were collected. Therefore, any hosts that tested positive for the same virus as their source pair was more likely to have acquired the virus from the source than from the community.

10.5.1.4 Fomites and Bioaerosols

All swabs collected from 19 toys prior to the first cycle of play tested negative. Subsequently, three toys (16%) tested positive soon after interaction; two (67%; 95%CI: ±0.09) were positive for one virus, and one (33%; 95% CI: ±0.09) had two viruses. One host clothing (5%) tested positive for RV post-interaction. Table 16 lists the viruses identified from fomites, and the associated rates of detection.

Bioaerosol samples were collected in the final two interactions during the study period. No viruses were detected during interaction on both occasions.

10.5.2 Sequencing

Eighteen adult hosts were determined to be shedding virus through the nose and throat on day 0, prior to interaction with paediatric sources. Seventeen shed RV and one shed FluA(H3). The host already infected with FluA(H3) did not continue to shed virus during the follow-up period and their source pair tested negative, hence, flu sequencing was not performed. On the other hand, 12 of the 17 (71%) hosts with prior RV infection continued to shed the virus throughout the follow-up period, and their source pairs also shed RV at interaction. RV samples from these 12 source-host pairings were, therefore, sequenced. Furthermore, all other RV-positive samples from sources and hosts were sequenced to determine RV types circulating in Leicester during the study period. Primers used in sequencing targeted the 5'-UTR of the RV genome. Sequence BLAST and phylogenetic analyses for sources and hosts are described below.

10.5.2.1 Paediatric Sources

A total of 77 paediatric source samples were sequenced; 61 nose-throat swabs and 16 hand swabs. Source RV type identification using BLAST analysis determined that RV-C infection was most common with sources, with 62% shedding virus through the nose and throat, and 56% having the virus on their hands. RV-C infections in sources were generally associated with co-infection with other RV types. Meanwhile, mono-infections were more often observed with RV-A (63%) than with either RV-C (15%) or RV-B (0%). Table 17 shows the number of source host, and fomite RV swab samples that were sequenced, the RV types identified

from the samples, the type-associated rates of infection, and the type-associated mono- and multiple infections.

Initial phylogenetic analyses of sequenced source samples were unsuccessful in constructing dendrograms that elucidated the clustering of source and host virus types, relative to reference virus strains in GenBank. Sequence data were no longer available for re-analysis.

10.5.2.2 Healthy Young Adult Hosts

A total of 73 samples from nose-throat, hand, and face swabs of 26 adult hosts were sequenced. In contrast to paediatric sources, RV type identification using BLAST analysis determined that RV-B infection was most common with hosts, with 63% shedding the virus through their nose and throats. However, RV-B was not identified on host hands and face, and 60% (41/68) of infections with the type were determined to be associated with multiple rather than mono-infections of RV. Table 17 lists the RV types identified from host nose-throat, hand, and face swabs, the associated rates of infection, and the numbers of mono- and multiple detections.

Initial phylogenetic analyses of sequenced host samples were unsuccessful in constructing dendrograms that elucidated the clustering of source and host virus types, relative to reference virus strains in GenBank. Sequence data were no longer available for re-analysis.

10.5.3 Viral Loads

Source virus copies/ mL from nose-throat swabs ranged between $<10^3$ and $>10^6$, depending on the virus. Viral load on source hands were lower, ranging from $<10^3$ to 10^5. Median viral loads for each of the viruses that were identified from source nose-throat and hand swabs, and for which quantitated RT-PCR were performed, are listed in table 18.

Host virus copies/ mL from nose-throat swabs ranged between $<10^2$ and 10^6, depending on the virus. Viral load on host hands were lower and did not exceed 10^3 copies / mL. On host face, viral load ranged between $<10^3$ and 10^5, while virus

on clothing was detected at $<10^3$ copies / mL. Median viral loads for the viruses that were identified from host nose-throat, hand, and face swabs are also listed in table 18. References that tested positive for EV and RV shed $<5.0 \times 10^3$ copies/ mL of each virus, while the reference with FluB shed 1.2×10^6 virus copies/mL of the virus.

Viruses on toys were detected at $<10^3$ copies / mL, which is also shown in table 18.

10.6 Virus Transmission Events

10.6.1 Large Droplet Transmission

Large droplet transmission of viruses that were shed by paediatric sources through their nose and throat and that resulted in their host pairs shedding the same virus through the nose and throat during follow-up days were observed with EV, RSVB and RV. The transmission of each of these viruses is described below.

10.6.1.1 Enterovirus (EV)

In total, 26 paediatric sources shed EV through the nose and throat, while 6 adult hosts shed the virus during the follow-up period. However, only one host had a source pair that shed the virus. The rate of EV large droplet transmission is shown in table 19.

The incubation period for host EV shedding ranged from one to seven days. Shedding was observed only once during the follow-up period. The median EV incubation period and shedding duration are listed in table 20.

10.6.1.2 Respiratory Syncytial Virus (RSVB)

RSV shedding through the nose and throat was observed in 27 paediatric sources. Source shedding comprised of six (22%) RSVA, twenty (74%) RSVB and one (4%) dual RSVA and RSVB.

Meanwhile, four adult hosts shed virus through the nose and throat during the follow-up period. Host shedding included one (25%) RSVA and three (75%) RSVB.

One of the hosts that shed RSVB had a source pair that was also determined to be shedding the virus during interaction. RSVB transmission rate is shown in table 19.

The incubation period for host shedding of RSVB was between two and six days. Shedding was observed on more than one occassion, and lasted until the end of the follow-up period. Table 20 lists the median incubation period and shedding duration for RSVB.

10.6.1.3 Rhinovirus (RV)

In total, 67 paediatric sources shed RV through the nose and throat, while 14 adult hosts shed RV during the follow-up period. Of the 14 hosts, 12 had source pairs that also shed the virus. Therefore, the RV large droplet transmission rate was 18% (12/67), when including only hosts that did not shed the virus prior to interaction on day 0.

In addition, four of the hosts that tested positive for RV on day 0 were determined by sequence analysis to have shed virus during the follow-up period that were of different type from what they shed on day 0, and that were ≥90% similar to the RV type shed by their source pairs. This suggests that transmission occurred and resulted in co-infection of hosts with virus from their source pairs. RV-A was the type most often transmitted, at a rate of 50%. The summary of RV large droplet transmission rates, in general and according to type, is shown in table 19.

The incubation period for host RV shedding ranged from one to seven days. Shedding was observed on more than one occasion and lasted until the end of the follow-up period. The median RV incubation period and shedding duration are listed in table 20.

10.6.2 Hand-to-Hand Transmission

Hand-to-hand transmission of viruses that were identified on the hands of paediatric sources prior to interaction on day 0, and that resulted in detection of the same virus on the hands of their adult host pairs soon after interaction, were observed with RSVB and RV. The transmission of each of these viruses is described below.

10.6.2.1 Respiratory Syncytial Virus (RSVB)

RSV was identified from the hands of 13 paediatric sources. Two (15%) sources had RSVA, ten (77%) had RSVB, and one (7.7%) had both RSVA and RSVB.

Two hosts had RSVB on their hands and had source pairs that also had RSVB on their hands. RSVB hand-to-hand transmission rate is listed in table 19. Transmission occurred within 20 minutes of source-host interaction.

10.6.2.2 Rhinovirus (RV)

RV was identified from the hands of 16 paediatric sources and three adult hosts.

Two out of three (67%) hosts tested negative for RV prior to interaction on day 0, and had source pairs that had RV on their hands. The third host shed RV on day 0. However, it was determined through sequence analysis that the virus shed by this host prior to interaction was of a different type from what was identified on their hands soon after interaction. Further, RV type on the third host's hands was ≥90% similar to the virus type identified on the hands of their source pair. This suggests that transmission has occurred. The rates of RV hand-to-hand transmission, in general and by type, are shown in table 19. RV hand transmission occurred within 15 minutes of interaction.

10.6.3 Self-Inoculation Following Hand Transmission

Following transmission of RSVB and RV from the hands of paediatric sources to the hands of their adult host pairs, the subsequent transfer of the same viruses from host hands to their own face was observed to have occurred in the study.

For RSVB, one of two hosts inoculated their face with virus, hence, a self-inoculation rate of 50%.

For RV, however, the results were a little more complicated. Two hosts had RV on their face. The first host transferred virus onto their own face with RV from their hands. The second host did not have virus on the hands. However, their source pair did. Sequence analysis of RV from the second host's face and their source pair's hands determined that they were ≥90% similar to each other. Further,

although this host was later determined to have been shedding RV on day 0, prior to interaction, the virus type shed was nonetheless different from the virus type identified on the face. These suggest that self-inoculation of host face with virus from source hands has occurred. It is likely, therefore, that all RV on the second host's hands were transferred onto their face.

In addition, EV was also identified on the face, but not on the hands, of the second host. The source pair had EV on their hands, and the host tested negative for the virus on day 0, prior to interaction. Therefore, EV could only have come from the source. It is likely that all EV on the second host's hands were also transferred onto their own face. The rates of host self-inoculation with RV and EV are summarised in table 19.

10.6.4 Fomite Transfer

The transfer of viruses that were identified from the hands of paediatric sources prior to interaction on day 0 and that resulted in detection of the same virus on toys and hosts' clothes, were observed with EV, HAdV, and RV. Virus transfer for each of these viruses is described below.

10.6.4.1 Enterovirus (EV)

All 19 toys were negative for EV prior to source-host interaction. One toy tested positive for the virus soon after playing commenced. The toy was played with by a source that was shedding EV and that also had the virus on their hands. The rate of EV fomite transfer is specified in table 19.

10.6.4.2 Human Adenovirus (HAdV)

All 19 toys were negative for HAdV prior to source-host interactions. One toy tested positive for the virus soon after playing commenced. The toy was played with by a source that was shedding HAdV and that also had the virus on their hands. The rate of HAdV fomite transfer is specified in table 19. HAdV transfer onto toys occurred within 20 minutes of interaction.

10.6.4.3 Rhinovirus (RV)

All 19 toys were negative for RV prior to source-host interactions. One toy tested positive for the virus soon after playing commenced. The toy was played with by a source that was shedding RV and that also had the virus on their hands. The rate of RV fomite transfer is specified in table 19.

One host clothing tested positive for RV, however, their source pair was RV-negative. This host was later determined to have been one of those shedding RV prior to source interaction on day 0. Therefore, it was presumed that contamination of host clothing occurred outside the interaction period.

10.7 Clinical Results

10.7.1 Paediatric Sources

Table 21 lists the proportion of paediatric sources presenting with any of the 15 systemic signs and respiratory symptoms included in the study criteria. The most common clinical manifestations in sources, as reported by their guardians, were cough (98%), difficulty-in-breathing (95%), and tachypnoea (87%). The median period from symptom onset to hospital admission was one day (IQR 1-2). The median interval from symptom onset to host interaction was also one day (IQR 1-2).

There were specific signs and symptoms experienced by sources that were determined to be of discrete statistical significance to the viruses with which they were infected. These signs and symptoms, the correlating viruses, and the associated *p* values, are listed in table 22.

10.7.2 Healthy Adult Hosts

All 111 adult hosts submitted completed diary cards. A total of 84 (76%) reported experiencing at least one of 21 signs and symptoms specified in the diary cards. Of these 84, 12 (14%) indicated clinical illness manifesting soon after interaction on

day 0. The proportion of signs and symptoms reported by hosts, and the actions taken to alleviate them, are shown on the host diary card in table 3.

The distribution of reported maximum symptom severity scores was as follows: 63 (75%; 95% CI: ±0.09) scored 1 (mild with no limitations to normal activity), 17 (20%; 95% CI: ±0.08)) scored 2 (moderate and some limitation to normal activity), and two each (2%; 95% CI: ±0.03) scored 3 (severe without needing medical attention) and 4 (incapacitating and needing medical consultation).

Influenza-like illness, defined in the study as mean daily symptom severity scores ≥6 that had durations ≥3 days, were reported by 22 (26%) hosts. This was extremely statistically significant ($p<0.001$). Figure 14 illustrates the incidence of ILI, the distribution of ILI severity indices, and the corresponding number of hosts in each index that sustained clinical illness for at least three days. Listed in table 23 are the specific respiratory symptoms and systemic signs that correlated with host ILI, the proportion of hosts with ILI that reported the signs and symptoms with severity scores ≥6, the proportion of hosts with ILI that reported the signs and symptoms with duration ≥3 days, and the associated *p* values.

Similar to the sources, there were specific signs and symptoms experienced by the hosts that were determined to be of statistical significance to RV or RSV that they either shed or had on their hands and face. The signs and symptoms, the proportion of RV- or RSV-postiive hosts that reported the symtpoms, and the associated *p* values, are listed in table 24.

10.7.3 Healthy Adult References

Seven (64%) out of the 11 adult references were symptomatic on the day of their visit to the research clinic. Of these, 57% (4/7) had symptom scores ≥6, with two reporting a maximum severity score of 2, the highest reported in this group. Two of the seven (29%) symptomatic references attempted to alleviate symptoms; one took oral analgesics, and the other sought primary care advice. Table 25 lists the signs and symptoms reported by the seven symptomatic references at presentation to research clinic, and the proportion that reported each parameter on the list.

10.8 Virus Transmission Based on Clinical Manifestations

There were adult hosts that developed ILI during the follow-up period, despite testing negative for all the target respiratory viruses, either by shedding, or by the presence of virus on their hands or face. Transmission was determined to have occurred when these hosts' source pairs tested positive for one virus, either by shedding or by having virus on their hands. Clinical manifestations were reported by RT-PCR-negative hosts whose source pairs had mono-infections of FluA(H3), HAdV, HCoV, hMPV, PIV1, PIV3,RSVA, RSVB, and RV. These viruses and their associated rates of symptomatic transmission, without shedding, are shown in table 19.

10.9 Virus Transmission Without Clinical Manifestations

Asymptomatic virus transmission by large droplet was observed in RV infections in this study. Three hosts that did not report any signs and symptoms in their diary cards during the follow-up period were, nevertheless, determined to have shed the same virus type as that shed by their source pairs. The rate of asymptomatic RV transmission is included in table 19.

10.10 Risk Factors Associated with Transmission

Other than virus capacity for transmission between symptomatic paediatric sources and healthy adult hosts, the different mechanisms of transfer involved, and the viral loads necessary for transmission to occur, risk factors associated with sources, hosts, and the environment were also measured in this study, to determine each of their contributions to the efficiency with which virus are passed on from human to human.

With paediatric sources, evaluated were the risks of transferring viruses on their own hands when shedding, and passing on viruses to their host pairs when shedding and having virus on their hands, viral loads that were greater than the median viral load measured for all children, and signs and symptoms of infection.

With adult hosts, evaluated was their risk of passing on infection to others. Finally, with environmental samples, evaluated were the risks of virus transmission associated with changes in temperature and relative humidity. The results on source, host, and environmental risk factors for transmission that were of significance, are described below.

10.10.1 Paediatric Sources

10.10.1.1 Source Shedding

The risk associated with symptomatic sources shedding virus through the nose and throat was determined to be of statistical significance with RV infections (p=0.037). The odds of source shedding virus when infected with RV were 97.1% (95% CI: 89.4 – 100), and the odds ratio (OR) was 5.5 (95% CI: 1.1 – 41). However, the risk of sources transmitting shed RV to hosts and that resulted in hosts shedding the virus as well, was not statistically significant (p>0.05).

10.10.1.2 Source Hand Contamination

In general, the risk of sources contaminating their hands with virus they were shedding was determined not statistically significant (p>0.05). However, when probing each virus individually, it was ascertained that sources shedding EV, PIV1, RSVA, RSVB, and RV were statistically more likely to contaminate their hands than when shedding other viruses. The calculated risks (R), risk ratios (RR), and statistical significance values for each of these viruses are listed in table 26.

Furthermore, when RSVB was detected on source hands, the risk to hosts of having virus on their hands and face was statistically significant. The risk of hosts developing respiratory symptoms and systemic signs was also statistically significant when sources had RSVB was on their hands. The calculated risks of RSVB on source hands, relative to virus on host hands and face, and host clinical manifestations, are listed in table 27.

10.10.1.3 Source Symptomatic Infection

When correlating source clinical manifestations with host symptom development, it was ascertained that interaction with symptomatic sources did not necessarily

result in host developing symptoms during follow-up (p>0.05). However, when probing each source symptom separately, it was determined that the risk of hosts developing ILI was statistically significant (p=0.024), when their source pairs presented with diarrhoea. The corresponding risk of this occurring was 54% (95% CI: 29 – 77), with an RR of 2.5 (95% CI: 1.3 – 5.0).

10.10.2 Healthy Young Adult Hosts

10.10.2.1 Host Pre-existing Immunity

In this study, baseline acute venous blood samples were collected to determine the pre-existing antibody profile of the host, using sera. However, serological analyses were not performed, due to the unavailability of testing methods during the study period. Therefore, sera will remain stored in laboratory freezers until such time as appropriate tests are developed for analyses.

10.10.2.2 Host Shedding

In probing the likelihood of symptomatic hosts shedding virus that they could then pass on to others, it was determined that when hosts had ILI, their odds of shedding virus, in general was 59% (95% CI: 39 – 77), with an OR of 3 (95% CI: 1 - 8). This was statistically significant (p=0.024).

Furthermore, the likelihood of hosts shedding virus in general, with symptom duration ≥3 days, was also statistically significant (p=0.012), with a 40% (95% CI: 29 – 53) odds of this occurring and an OR of 0.3 (95% CI: 0.1 – 0.8).

Meanwhile, when probing individual viruses, it was determined that the odds of RSVB-positive hosts developing illness were statistically significant (p=0.011), at 95.5% (95% CI: 76.5 – 100), with an OR of 8.56 (95% CI: 1.46 – 188). However, RSVB infection was not correlated (p>0.05) to host either reporting symptom severity scores ≥6 or symptom durations ≥3 days.

10.10.3 Environmental Factors

In this study, environmental factors measured included temperature and RH in the interaction rooms. These results are shown in table 28. However, since individual readings remained generally uniform during the course of the study, no further analyses were performed relative to temperature and RH.

11 Discussion

11.1 The TraVerse Method and The Forgotten Pandemic

The common cold is the forgotten pandemic.[3,36,37] It has been a documented burden on the health and economic wellbeing of global populations for generations, yet its cure and measures for its control and prevention have yet to become successful.[37] Success remains elusive, partly because there persists a gap in knowledge of its transmission, particularly with regard to the contributions of causative viruses, the human source and host, and the environment, to the spread of disease. Landmark studies and PCR [10,13,16,23,35,95,124,411,749] provided current transmission models, however, supporting data are deficient in three ways: 1) humans have not been used in natural transmission studies, 2) studies on healthy young adults are limited, and 3) there is a lack of authenticity in study settings used. A human model is critical to natural transmission studies because evidence indicates that respiratory infections in experimental conditions may differ from what occurs in actuality.[750-752] Studies involving healthy young adults are necessary because recent findings show that severe infection in this population is more common than originally thought.[31,49,612,616,651,753] When they are severely affected, there is an increased risk to global health, infrastructure, and economy, because it is this demographic that is in the workforce, sustaining social standards. Finally, study settings need to be as near to real-life situations as possible, so that appropriate methods of infection control and prevention are effectively implemented. This study attempted to address these deficiencies by using the TraVerse method of natural, human-to-human transmission.

With the TraVerse method, symptomatic paediatric patients, who are known to shed respiratory viruses at high levels, acted as the sources of viruses that they then passed on to healthy young adults acting as virus hosts. The human-to-human transmission of viruses from source to host transpired during 30-minute simulations of interactions that commonly occur between the patient and their medical teams during clinical consultations, in a fully functioning setting. Paediatric sources and adult hosts remained within three feet of each other during interactions, to simulate direct, large droplet transmission. Hand contact between

source and host during clinical examinations, followed by hosts touching their own face, mimicked indirect virus transfer by hand contamination and self-inoculation. Exchanging toys during playing, and contact during clinical examinations simulated virus transfer by fomite. Finally, collecting air samples with a portable bioaerosol sampler, during the entire length of interactions, assessed small droplet aerosol transmission. Functioning hospital wards were the controlled settings where interactions occurred because these places and their staff are the front lines, particularly in pandemic situations.

The emergence of SARS and the re-emergence and spread of avian influenza A(H5N1) provided the impetus for WHO to mandate the creation of pandemic preparedness plans by every country.[754] In light of the history of avian and swine influenza pandemics occurring several times each century, with four pandemics in the past century and one already at the beginning of this century,[140,170] pandemic preparedness plans have understandably been focused on the next flu pandemic occurring. However, the rapid spread and virulent infections observed with SARS and MERS-CoV,[42,419,444,495] and the recent deaths associated with emerging strains of HAdV,[523,542,543] demonstrate that other respiratory viruses implicated in common cold infections are just as likely as flu to cause the next pandemic, but these viruses have been overlooked. This research addressed the oversight by evaluating all known respiratory viruses identified during the three-year study period, using the more sensitive PCR method of diagnosis.

It is also predictable that there is a renewed, avid interest in research on virus mechanisms of transmission to make sure that current pandemic plans are practical. However, although developed countries apportion billions of dollars towards pandemic preparedness and response, only a very small percentage of government budgets go towards respiratory disease research.[3,36] In the UK, this is further compounded by the reduction in financial allocation by private interest groups, such as the Medical Research Council, Wellcome Trust, National Institutes of Health Research and Cancer Research UK, from where two-thirds of respiratory research bursaries come.[36]

The common cold was not the forgotten pandemic on this occasion however, because this study on the fitness for purpose of the TraVerse method had a financial sponsor who understood that respiratory disease transmission research is important. A larger budget would have accomplished more; however, this research has the largest number of participants in transmission studies, and it successfully demonstrated that the first real-life based method of transmission is fit-for-purpose, which suggests that the small budget was, nevertheless, put to good use.

11.2 RT-PCR Diagnosis of Viral Infections

Pivotal to the study of the common cold are the methods of identifying pathogens associated with infection.[755] These methods include virus culture in living cells, detection of virus antigen using host antibodies, and confirmation of host infection with serology. However, the sensitivity and rapidity of diagnoses with these methods are inadequate in clinical settings.[755,756] For example, in children with LRTI, overall sensitivity in virus detection does not exceed 40%,[755] and diagnoses are often confirmed long after patient discharge or death.

Respiratory illness is the primary reason for seeking clinical consultations by all age groups.[756] During peak seasons, respiratory illness causes severe disease and increased mortality.[756] Disease diagnosis is difficult because several viruses cause common cold symptoms, clinical manifestations of disease are relatively similar for all viruses, and infection with more than one virus generally occurs, particularly in children.[756] Cell culture is the gold standard for identifying respiratory viruses. This method is sensitive and specific in differentiating viruses, however, it can only identify Flu and RSV with high sensitivity, it cannot differentiate viruses on the subtype level, and it restricts the types of samples used and the conditions of sample handling because live virus is needed.[552,755,757]

In the 1990s, newly developed molecular diagnostic methods enabled virus identification based on their genetic material.[552,755,756,758] PCR is a molecular amplification method that exponentially generates multiple copies of virus genetic

material, through a cycle of heating and cooling. Specific primers, designed to bind to conserved regions of a gene, start or 'prime' the process, and thermo-stable enzymes keep the cycle going. PCR transformed respiratory diagnostics into something of more clinical value, by increasing the sensitivity in virus identification and the speed in disease diagnosis.[755] Benefits of PCR include, promoting a better understanding of viruses associated with LRTI, managing nosocomial infections with viruses identified in high-risk wards, enabling the stewardship of antibiotics when bacterial infection is not detected,[552] guiding therapies based on virus genotype and on whether mono- or multiple viruses are present,[755,756,758,759] and establishing asymptomatic infection.[755]

On the other hand, challenges associated with PCR include, the clinical utility of result interpretation,[552,755,758] the impact of rapid identification on clinician behaviour,[552] and the nature of the samples used for diagnostics.[552,755,757] Identifying viruses during asymptomatic illness and multiple infections makes it difficult to assign causality to clinical manifestations, raises the issue of false-positive tests, and challenges clinician ability to interpret results.[552,755,758] To address these concerns, Jartti (2013) argues that infection correlates with PCR positivity because the method provides results in an expected way; it is more sensitive in detecting symptomatic than asymptomatic infection and in identifying asymptomatic infection in patients with underlying chronic illnesses than in the healthy.[755] Furthermore, since PCR is considerably more sensitive than other diagnostic methods, then the numbers of positive samples should also increase.[755] Meanwhile, Pavia (2011) suggests that clinicians interpret PCR results in the context of virus epidemiology, the likelihood of infection, and patients' symptoms and overall health profile.[552] Pavia further suggests that clinician action after diagnosis and result interpretation is just as important in patient management and increases the likelihood of a positive outcome.[552] Actions may include the use of antivirals, avoidance of antibiotic prescribing, and implementation of infection control practices.[552,758]

Finally, there is concern that upper airway samples used in PCR do not accurately represent what naturally occurs in the lower airways. In particular, that positivity in upper airway samples may prove not an active LRTI, but rather a resolving

URTI.[552,755] Jartti et al. (2013) brought attention to studies that identified viruses from bronchoalveolar lavages and lung biopsies, which suggest that infiltration of distant bronchioles has occurred, thereby, demonstrating that virus detection rates in both upper and lower airways were similar.[755] However, although this argument supports the use of upper respiratory samples to diagnose lower respiratory disease, there are further concerns about the nature of the samples themselves.

The likelihood of obtaining a correct diagnosis, by any diagnostic method used, is dependent on clinical sample quality.[760-762] A good quality sample is one collected and transported properly to the laboratory; these are the pillars of a rapid and accurate respiratory disease diagnosis.[760] Nasopharyngeal aspirates (NPAs) and nasopharyngeal washes (NPWs) are the best quality specimens because they are able to capture many ciliated epithelial cells during the procedure, hence, their standard use in clinical diagnosis[760-762] However, collecting these samples is time-consuming, requires specialised equipment and technically trained operators, generates aerosols, and inflicts pain on the patient.[760,761] It is, thus, suggested that the best alternative to NPAs and NPWs would be one that balances obtaining a good result with available resources, staff abilities, the risk of infection, and patient tolerability.[761] One such alternative is the flocked swab designed specifically for the collection of respiratory samples.

The flocked swab differs from the standard nasal swab, in that it has perpendicular flocked fibres that enable the soft brushing of clinical samples from the patient's nose, and the easy release into standard laboratory VTM.[760] Several studies have compared flocked swabs in capturing virus particles and in the rates of detection with PCR, against NPAs and NPWs. Chan et al. (2008) determined sensitivities of 100% for Flu and 92.3% for RSV in paediatric patients.[761] In terms of viral loads, NPAs were found to have higher titres. This was statistically significant with RSV infections, where there was a nine-fold difference observed. Nonetheless, flocked swabs were better than NPA because they created lesser aerosols, were better tolerated by children, and preferred by the parents.[761] Abu-Diab et al. (2008) established similar findings, with flocked swab sensitivity rates of 100% for Flu and PIVs 1, 2 and 3, 98.4% for RSV and 88.9% for HAdV. [760] Their findings on

epithelial cell capture were likewise similar to Chan et al. However, they observed that, although NPAs collected more cells than flocked swabs, swabs were just as capable of collecting and releasing cells that were detectable by diagnostic methods.[760] Moreover, approximately 98% of nurses recommended flocked swabs because they were less traumatic for patients and required less time and staff training. Nurses also observed a higher rate of children crying during NPA collection, than during flocked swab sampling.[760]

A correct diagnosis is also enhanced by the PCR method used. The genetic material of most respiratory viruses is RNA, hence, it is first transcribed into complementary DNA (cDNA), through a process called reverse transcription (RT). RT is either performed separately or in the same tube as the PCR reaction, which is known as the reverse-transcription PCR (RT-PCR) method.[755] In cases where a rapid result has implications for antiviral use, such as in flu, obtaining a diagnosis in real-time might be helpful. For these, a real-time RT-PCR method is useful.[755] On the other hand, in cases where patient recovery needs close monitoring, quantitative RT-PCR measures the viral loads for sequential samples.[755] Finally, for patients that would benefit from testing for infection with multiple viruses rapidly and, hence, would need to be tested for at the same time, a multiplex RT-PCR would suit.[755] Most diagnostic laboratories routinely screen for multiple viruses at the same time, which is why multiplex RT-PCR is more often used in these facilities.[755]

In this study, flocked swabs were used to collect samples and quantitative and qualitative, multiplex, real time RT-PCR was used for virus identification. The PCR method is more cost-effective and timesaving than doing several single target reactions, uses less sample, provides a mechanism of assay control in using positive samples as internal controls, and enabled gene sequencing of RV.[755] The challenges with this method, however, include, the complicated technical design that is necessary, the absolute optimisation required, and the highly sophisticated instrument that is essential.[755] The PCR methods used in this study have the added benefit of measuring viral loads either in relative or exact values, which can then be used to extrapolate disease progression and impact of therapy. However, it requires dilutions of standards of known concentrations and, if the lowest

standard dilution is higher than the instrument detection limit, then this decreases the assay sensitivity.[755] RT-PCR results suggest that assay sensitivity in this study was not very much affected. Overall rates of shedding were 93% in paediatric sources and 22% in healthy young adult hosts. Assay sensitivity in samples from source was comparable to those reported in previous paragraphs when using flocked swabs. On the other hand, there are no known published rates in naturally infected healthy adults at the time of writing. Therefore, RT-PCR assay sensitivity in adults was a novel finding with the TraVerse method.

11.3 Fitness of the TraVerse Method

The first recorded study on the mechanisms of virus transmission using human subjects was that of Russian scientists in 1936.[14] Five volunteers were artificially inoculated with aerosolized flu virus isolated from patients in Leningrad, during the highly fatal flu epidemic of that year. This study determined that the flu virus could cause symptoms of the disease and effectuate an immune response.[14] This was followed, unsuccessfully, by Sir Christopher Andrewes' use of human volunteers at the CCU in the 1940s.[763] During this time, the many pathogens of the common cold have not yet been identified, thereby impeding efforts to cause disease in volunteers.[750] Things changed for the better upon the discovery of rhinoviruses in 1956, which accounts for many of the models of transmission devised in the study of RV.[750]

The first RV model was that of Gwaltney, et al. (1980),[11] which elucidated that RV is not transferred by aerosol, as shown by the failed infection of hosts separated from sources by double wire partition. Subsequent to this was D'Alessio et al.'s studies involving volunteers housed together, kissing couples, and married partners.[10,100] Although their results showed that kissing is not a high-risk activity for passing on respiratory infections, it was not possible to draw any other verifiable conclusions from them, due to poor experimental design and the possibility that many other routes of transmission may have occurred at any one time.[750] Hendley et al. (1973) determined RV transmission by host hand contact

with source hands, contaminated objects, and environmental surfaces in their experiments involving 15-second finger contact between sources and hosts.[96] The more recognized Antarctic hut model or miniature field trial (MFT) by Meschievitz et al. (1984) followed,[13] which emulated Holmes et al.'s (1976) RV experiments on volunteers living in isolated huts in the North Pole.[101] This model involved secluding sources and hosts in a small room for 5 hours and up to several days, where they interacted with each other by playing games using board, video, or cards. A more recent adaptation of these trials was also used in volunteer studies by Killingley (2011, 2012), which focused on Flu transmission.[12,358] These models using the seclusion of volunteers altogether established the linear relationship between the rates of virus transfer by aerosol, hand contact, and fomite and the amount of time of exposure by a susceptible host to an infected source.[13,750]

Data generated from the different models of transmission provided much knowledge, however, the artificial inoculation of volunteers limits their application because it is not an accurate illustration of what occurs in natural infection. For example, models for large droplet transmission used laboratory grown virus types at highly concentrated titres when, in nature, there could be several types or even different infecting viruses present at the same time, in varying concentrations, and that may slightly differ from wild type.[750] Meanwhile, hand transmission models relied on intentional handling of experimentally infected objects and surfaces immediately after contamination, when it is more likely that several steps and circumstances would lead one to handle an object at the exact times when the virus is more highly transmissible. [750] Finally, MFTs consecutively exposed hosts only to severely ill sources continuously for several days, while this is highly unlikely in natural circumstances.[750]

To overcome the shortcomings of inoculation studies, Gwaltney et al. (1978) endeavoured to more definitively infer the causality of virus transmission by applying predictive performance. The rationale was that interventions against transmission that are appropriate to the virus and its mechanisms of transfer result in diminished rates of infection in the susceptible population.[750,764] These studies proceeded from 1979-1982, when 50 different households in Virginia, U.S. implemented interventions for hand transmission, with successful results.[750]

Infections in these studies were considered natural, rather than artificial, because colds were not contrived by artificial inoculation with virus.

TraVerse differs from these models of transmission in that, it included all viruses relevant to the common cold that is identifiable by molecular methods, and it mimicked situations and circumstances in functioning secondary and tertiary paediatric facilities in the UK. Specifically, TraVerse mimicked toy playing with hospital play specialists, clinical examinations with medical staff, and face-touching with unwashed hands. Its contributions include the elucidation of the rates and mechanisms of natural common cold virus transmission, recognition of the risks of transmission by specific mechanisms in healthcare settings, and awareness of the consequences of viral infections in healthy young adults, all of which, are relevant in the prevention and control of pandemics. TraVerse was fit-for-purpose, providing virus transmission occurs by identifying the same virus or virus type from both the sources and their host pairs within the follow-up period, or that the hosts develop ILI for at least three days. The results of this study illustrate that natural, human-to-human virus transmission occurred with the TraVerse method. Observed in the study were the large droplet transmission and self-inoculation with EV, RSVB, and RV, hand transmission with RSVB and RV, and fomite transfer of EV, HAdV, and RV. Meanwhile, ILI without virus shedding was observed in hosts whose source pairs shed HCoV1, FluA(H3), RSVA, RSVB, and RV. These results, therefore, suggest that TraVerse was fit-for-purpose.

Regrettably, transmission that resulted in hosts shedding Flu, HCoV, hMPV, and HPIV was not observed. This could be because the university holidays and exam periods coincided with peak respiratory season, sources were either in the prodromal or recovery phases of infection, hosts shed at levels not detectable by RT-PCR, hosts shed at levels lower than the quantitative standard value of 10^3 virus particles/mL, or aerosol transmission occurred, which was inadequately evaluated in the study.

11.4 Virus Transmission Rates and Mechanisms

11.4.1 Rhinoviruses (RV)

11.4.1.1 Large Droplet Transmission

In this study, the overall rate of natural large droplet RV transmission was 24%, with 16 hosts shedding the same RV type as their source pairs. RV-A was the type most often transmitted, either by itself (12%) or with RV-C (9%). Meanwhile, RV-B transmission did not occur. Three other studies between 1970 and 1984 established the role of large droplet transmission in RV infections using different methods, and with varying rates of success. In 1970, Douglas et al. used the traditional method of directly inoculating the nasal passages of volunteers with RV drops.[765] By this method, the infection rate was 50%, with as little as 0.1 $TCID_{50}$ of the inoculum. However, they used a virus strain of unspecified type; hence, virulence did not correlate with the reported $TCID_{50}$. In 1976, D'Alessio and colleagues used childless married couples as volunteers; one acted as RV source and the other as host. [100] They determined transmission rates of 33% and 41% depending on the virus type used. However, host infection occurred only on four conditions, the inoculum had high virus titre (≥1000 $TCID_{50}$), the source had the virus on their nose and hands and was moderately symptomatic by the study criteria, and the couple spent ≥122 hours in their home together. Moreover, since other modes of virus transfer could also have occurred in their study, it was difficult to infer the exact route of transmission.[750] D'Alessio's team also exposed hosts to infected sources through kissing.[10] The throat washes of hosts contained RV; however, transmission occurred in only one of thirteen events (8%). They inferred this as confirmation that oral inoculation is an inefficient mode of RV transmission, and that kissing is a relatively safe activity.[10] They proposed further that natural large droplet transmission is difficult to simulate during short-term exposure, possibly because high titres are necessary for virus transfer to occur.[10] By contrast, the TraVerse study established that RV large droplet transmission does occur in natural infections, within a mean exposure time of 23 minutes, and at source median virus shedding titre of 10^4 particles per millilitre.

11.4.1.2 Hand-to-Hand Transmission

The TraVerse method also established an overall natural hand-to-hand RV transmission rate of 19%. Similar to RV large droplet transmission, RV-A was most often transmitted (13%). Meanwhile, RV-C only transmitted when co-infecting with RV-A (6.3%). Experimental models of transmission determined varying rates of RV hand transmission, depending on virus type, the titres used, and the duration of exposure of hosts to the infected sources. Pancic et al. (1980) estimated a mean hand-to-hand transmission rate of 6.7% when infecting source hands with 0.05 mL of mucus containing up to 50,000 plaque-forming units of RV of unknown subtype.[766] Reed (1975) established a transmission rate of 17% when hosts rubbed dried RV inoculum on source fingers or hands for 15 seconds; this rate doubled with damp inoculum.[767] Despite these rates of transmission, Reed (1975) concluded that infection of the host by hand-to-hand transmission is unlikely unless the host is in close contact with a source that is actively shedding virus, such as an acutely ill child.[767] Meanwhile, Gwaltney et al. (1978) determined a rate of 71% during 10-second hand contact of the host with source hands inoculated with nasal secretions. [24] The range of these rates of transmission illustrates the difficulty in correlating data from experimental infection studies with natural infections, because the virus concentrations used are estimates of what occurs naturally, and not real-life observations. Furthermore, the speed with which host infection occurred with TraVerse cannot be compared with the 10-second findings of Gwaltney et al. (1978), and the 15-second experiments of Reed (1975) because hosts in this study were swabbed at the end of the interaction, and not at specific time points. Therefore, it is suggested here that RV hand-to-hand transmission occurs within 15 min. to 25 min. of hand contact between the virus source and host.

The TraVerse method used copy numbers to illustrate virus concentrations, which do not correlate with plaque forming units and $TCID_{50}$ values[768] Nevertheless, this study established that hand transmission occurred with a median viral load of 10^3 RV particles / mL, which was one to two logs less than in large droplet transmission. This suggests that RV transmission by hand is more efficient than by large droplet, conceivably because of the direct infection of the eyes and nose with

the hands. This is supported by Gwaltney and Hendley (1978) in their study on RV fomite and aerosol, which established that the primary mechanism of RV transfer is more likely by fomite than by air, particularly when hands containing the virus comes in contact with the eyes and nose.[764] This study also determined that there was a 21% risk of sources shedding RV and infecting their own hands and that they were five times more likely to do so than if they were not shedding the virus. This, therefore, provides a possible explanation for the efficient hand-to-hand transfer of RV.

11.4.1.3 Transmission by Self-Inoculation

Two-thirds (67%) of the hosts in this study on whose hands RV transferred also inoculated their faces with the virus. RV-A was the type most often transmitted by self-inoculation (50%), while RV-C only transmitted when co-infecting with RV-A (50%). Adults touch their faces often, enough to pose the risk of infecting themselves and then passing on diseases to others.[96,769] To discourage medical students from this habit and encourage hand hygiene, Kwok et al. (2015) conducted a behavioural science study on face touching by fifth-year medical students.[769] Within a one-hour period, 90% of students touched their face; 44% of which touched their mouth, nose, and eyes.[769] Hendley, et al. (1973) established that, when touching one's nose and eyes with RV-infected hands, 36% of volunteers became ill, [96] while Reed (1975) determined that a moderate infectious dose of virus (88 $TCID_{50}$) is all that is necessary for a self-inoculation rate of 20% to occur if the finger is still damp with the virus.[767] Therefore, the rate of natural infection by self-inoculation in this study was higher than rates published from studies on experimental infection with RV.

11.4.1.4 Fomite Transmission

Using the TraVerse method, RV transfer from sources to toys only occurred with co-infections of RV-A and RV-C, at a rate of 7.7%. This rate is lower than that reported by Reed (1975) on the indirect contact transmission of RV,[767] wherein only 14% of objects contained the virus even though they were touched not too long beforehand by experimentally infected sources. Reed (1975) further determined that rubbing promoted RV transfer, but that virus infectivity

diminished by 40% to 99% after surface drying occurred. Therefore, they concluded that RV transmission was unlikely to occur through contact with surfaces touched by infected sources, particularly once the surface dries.[767]

This study further determined that RV fomite transfer occurred even at viral loads $<10^3$ copies / mL but, that subsequent handling of contaminated fomites by the host did not promote infection with the virus. This was supported by Reed's (1975) conclusion that RV transmission was unlikely to occur through contact with contaminated surfaces unless virus titres are high.[767] Meanwhile, Hendley et al. (1973) suggested that, in naturally occurring colds, rubbing of surfaces contaminated with RV promoted infection with the virus, at a rate of 36%, because rubbing increases the amount of virus transferred on the hosts' hands.[96] Finally, Winther et al. (2011) demonstrated that 22% of fomites deposited on surfaces one hour before contact were transferred on host hands, however, the rate dropped dramatically to 3% after 24 hours, and then to zero not long after. They concluded that RV transfers from objects and surfaces to the hands; however, infection decreases 24 hours after the virus is deposited on fomites.[770]

By contrast, Jennings and Dick (1987) argued that, although fomite transmission is possible in experimental RV inoculation, it is not a common occurrence in natural infections.[771] In their MFT model of transmission, infection of hosts did not occur despite exaggerated amounts of natural cold secretions on fomites handled by volunteers for 12 hours. Instead, they proposed that the primary mode of RV transmission is via aerosol, which demonstrated a 50% rate of infection in their MFTs.[771] However, exposure to aerosols for at least 200 hrs. and viral loads ≥1000 $TCID_{50}$ were necessary for infection to occur. They concluded that fomite studies by other groups did not establish RV transmission because either aerosol exposure was not long enough or virus titres were too low. [771]

This study was not able to establish RV aerosol transmission due to the delayed implementation of bioaerosol sampling. Although had sampling started sooner, perhaps the findings would remain the same, since the interactions lasted less than 200 hrs. and the virus titre on toys were too low. It is possible that an increased viral load by either source shedding of higher titres or host rubbing of

fomites could have resulted in transmission from the toys to the hosts. In real-life settings, however, runny nose and spittle are generally thought of as a nuisance and as unhygienic; hence, are often wiped off with tissue or one's sleeve (or arm, in children). Furthermore, perhaps the only reasons one has for rubbing surfaces are to clean them or for good luck. Realistically, therefore, aerosol transmission of RV may just be observed in families or in settings where virus sources and hosts are in very close physical contact for long periods, while RV transmission from fomites to hosts may just be due to bad luck.

11.4.1.5 Clinical Manifestations

Advances in molecular diagnostics have resulted in standard laboratory respiratory testing that includes RV and, by extension, in the timely clinical identification of RV infections. However, clinical data availability, relative to an RV diagnosis has not kept pace with the rapid identification of RV strains, thereby impeding attempts at correlating RV type to clinical manifestations.[71] The use of the TraVerse method determined that symptomatic children were more likely to have systemic signs than respiratory symptoms when infected with RV. Furthermore, that the systemic signs that significantly correlated to RV infection in this group were febrile illness and diarrhoea (table 22). The negligible upper respiratory involvement is supported by the finding that, although children develop a cough and SOB during RV infection, other viruses are just as likely to cause these symptoms. Therefore, respiratory symptoms alone are not appropriate predictors of RV infection in children.[71]

Studies suggest that RV is generally found in the nasopharynx, close to the throat, where it is cleared by mucociliary mechanisms. Ironically, the virus avoids clearance and ends up on the inside of the nose through blowing one's nose.[9,772] Moreover, unlike Flu and RSV, RV is not generally associated with epithelial cell damage in the upper respiratory tract; hence, even if the virus migrates to the nose, any cytopathology would not be distinguishable.[9,91,773] Nevertheless, RV infection promotes secondary bacterial infection and innate immune response, by interfering with the barrier function of epithelial cells.[9,774] In this study, the mean age of children was 26 months (table 14); therefore, it is not likely that they would

have known how and when to blow their nose. Therefore, it is suggested here that the absence of an association between RV infection and upper respiratory symptoms in children in this study was due to the virus remaining in their throat. However, the viruses that escaped clearance by the mucociliary escalator may have activated the innate inflammatory response, which manifested as systemic signs.

In contrast to sources, both respiratory symptoms and systemic signs were indicative of RV infection in adult hosts in this study (table 24). More importantly, lower respiratory tract involvement is notable, which presented as productive coughs. Furthermore, fatigue was common in the host population, which is a possible explanation for work absences during infection. Symptoms of RV infection in healthy young adults are not well understood, and literature is contradictory. Studies involving artificial virus inoculation confirm that LRTI results from RV infection.[9,775] However, they also suggest that infection by one type immediately prevents reinfection with the same type and co-infection with another RV type for two to sixteen weeks.[93,752,776] By contrast, Rosenbaum et al.'s (1971) study on natural RV infection in adults in the military demonstrated consecutive infection within two days.[752,777] RV-B was the type most often identified in hosts in this study, which manifested mostly as a co-infection with other RV types than as a mono-infection (table 17). Further, co-infection of hosts with a virus type acquired from their source pairs resulted in symptoms within one day of infection and shedding of the virus after 5 days. Therefore, these suggest that clinical findings on RV infection in adult hosts, using the TraVerse method, agree more with the results obtained by Rosenbaum et al. (1971) than with other studies.

The period from RV infection to clinical manifestation in this study was between three hrs. and five days, while symptoms lasted from one day through to the duration of the follow-up period. Gwaltney (2002) suggested incubation periods from 10 to 16 hours, peak infection at 2 to 3 days post-infection, and a typical duration of one week, but is longer in 25% of cases.[778] Meanwhile, Lessler (2009) determined that most experimental studies reported incubation periods between two to four days; however, these were during peak symptom development and may, therefore, be biased. He instead estimated that 95% of cases developed

symptoms between four to five days post-infection.[751] The benefits of using the TraVerse method were that the exact days of infection were known, and symptom diary cards provided the specific dates and times of clinical onset and duration. Therefore, considering the reported mean values in context, this study suggests that, in natural RV infection of healthy young adults, most will develop symptoms within two days after infection, and recover within five days.

Finally, Peltola et al.'s (2008) studies involving households with children, which determined that 16% of adults had asymptomatic carriage support this study's finding that 15% of healthy young adult hosts with RV infection was asymptomatic. By contrast, Camargo et al.'s study involving healthcare workers only ascertained an asymptomatic infection of 3.6% in adults,[779] while Johnston and colleague's (1993) research on immunocompetent adults established a 4% rate.[780] They attribute the higher rates of asymptomatic carriage seen in adults to nosocomial, and not community-acquired, infection. However, the opposite was true in this study as, although the asymptomatic rate was 15% for all RV-positive hosts, the rate dropped to 5% when including only those whose source pairs had RV infection (table 19); presumably, because most asymptomatic host RV infection in this study was associated with host shedding on day 0, before interaction with sources, which suggests a community-acquired infection.

11.4.1.6 Novel Findings on RV Transmission Using the Traverse Method

In this study, there was a distinct predominance of RV-B infections in healthy young adults, the rate being 63%. This is a novel finding, as far as the researcher is aware. By contrast, the rate of RV-B infection in paediatric sources was only 6.6%, which was a dramatic drop from RV-C at 62% and RV-A at 31%. Furthermore, although RV-C was the predominant infection in sources, it was not as easily transmissible to healthy adults (13%) as RV-A (50%), unless dual infections with RV-A occurred (38%).

Several studies have demonstrated that RV-C infections are relevant in wheezing and severe infections requiring hospitalisation in paediatric populations.[781-783] A study by Cox et al. (2013) was the first to realise the contributions of each RV type

to paediatric ED consultations and hospitalisations.[784] Their findings indicated that, although there was an equal likelihood of ED consultations in children infected with RV-A and RV-C, hospitalisations were twice more likely in those with RV-C. By contrast, children with RV-B were three times more likely than those with either RV-A or RV-C to require ED consultations. There were not enough hospitalisations due to RV-B to establish its relevance in admissions.[784] Meanwhile, research by Rahamat-Langendoen et al. (2013) suggests that, although RV-B was the least identified infection in hospitalised children, it was nevertheless implicated in 40% of nosocomial infections and in diseases that required extra oxygen therapy in this population.[71] Therefore, hospitals are conducive to RV-B transmission, particularly in instances of asymptomatic carriage or subclinical infection. However, severe infections occur only in those hospitalised due to underlying conditions.[71]

The findings that RV-B is not commonly associated with hospitalisations in paediatrics agree with this study's. Meanwhile, nosocomial RV-B transmission was not observed, possibly because the numbers were not large enough or, more than likely, because the hosts were healthy young adults and not children. Moreover, none of the adults with RV-B were ill enough to need medical consultation, hospitalisation, or oxygen therapy. Therefore, it is suggested here that healthy young adults are more susceptible to RV-B than either RV-A or RV-C, but the associated illnesses are relatively mild so as not to require consultation, hospitalisation or supportive oxygen therapy. Furthermore, RV-B is not easily transmissible between symptomatic paediatric sources and healthy young adult hosts; this could be because there were not enough hospitalised sources infected with RV-B in this study.

11.4.1.7 Summary of RV Findings

To summarise the findings on RV transmission using the TraVerse method, RV large droplet transmission occurred within 23 minutes of close interaction of healthy adults with symptomatic children infected with RV. This finding was in contrast to studies that used the artificial inoculation of adults, which suggested that infection with large droplet is not an efficient mechanism for RV transmission.

Hand-to-hand transmission of RV also occurred in this study; it required less virus titre for transfer to occur and was thus, the more efficient mechanism for RV transmission. The reasons provided for this efficiency were because the adult hosts directly inoculated their nose and eyes with the virus, and the paediatric sources were five times more likely to contaminate their hands when they were shedding RV through the nose and throat.

Fomite transmission occurred at a rate lower than that reported from studies that used artificial inoculation, more than likely because the toys were not touched for long periods by the sources.

Clinically, systemic signs were more common than respiratory symptoms in children with RV infection. This is likely because the mucociliary clearance of the virus activated an immune response, which manifested as systemic signs in the children. In the adults, cough and fatigue were the signs and symptoms that significantly associated with RV infection, which can result in missed days at work. A small proportion of adults had asymptomatic RV infection, likely due to a community-acquired illness.

Finally, a novel finding using TraVerse was that RV-B is more common in adults than in children, but is associated with mild illness. RV-A is more transmissible than either RV-B or RV-C, and RV-C is most often transmitted in co-infection with RV-A.

11.4.2 Enterovirus (EV)

11.4.2.1 Large Droplet Transmission

In 2008, the International Committee on Taxonomy of Viruses removed the genus *Rhinovirus* from the list of classifications to include RV species in the *Enterovirus* genus.[785,786] This was necessary because RV and EV are not genetically or structurally distinct, and RV-B and EVs are more genetically related to each other than each is to RV-A.[786] Thus, EV in this study refers to the species that primarily

colonise the gastrointestinal tract, but that also have respiratory and CNS tissue tropisms.[786]

Using TraVerse, a 4% rate of EV large droplet transmission occurred; however, statistical analyses suggest that symptomatic paediatric sources passing on EV infection to healthy adult hosts by the large droplet route may just have occurred by chance in this study.

11.4.2.2 Transmission by Self-Inoculation and by Fomite

Meanwhile, self-inoculation of the virus occurred on one occasion, which equated to a rate of 100% and fomite transmission occurred at a rate of 20% (table 19). This correlates with the statistically significant finding in this study that the sources were 40 times more likely to have EV on their hands when they are shedding the virus (table 26). Supporting this is the observation that EV enters the host through the faecal-oral route; the virus then replicates in the gut where they are resistant to body temperature and gastric acid.[786] EV on the hands of sources suggest that at some point during the interaction, they must have placed their hands in their mouth, which is typical behaviour in this age group.

Mouthing is the childhood behaviour of infants and toddlers placing objects, fingers, toes, or food in their mouth as they go through the developmental process. It is a means of exploring their surroundings and alleviating the irritations associated with teething, although it also poses the risk of ingesting contaminants present in the environment.[787] There is no evidence that frequency of mouthing associates with a child's gender. However, there is a known correlation between frequency of the practice and age at mouthing;[787] children aged up to 24 months mouth the most often, at 73 episodes within an hour.[787] The preference for what to place in their mouth again depends on the age. Those aged 3 – 6 months prefer fingers, followed by toys. At 6 – 12 months, the order of preference goes from toys, followed by other objects, and then fingers. At 12 – 18 months, non-toys and then fingers. Finally, at 18 – 60 months, it is all fingers.[787,788] In this study, the mean age of EV-positive children was 33 months, and the median age was 26 months; thereby, placing them in the finger-mouthing group. Moreover, the one source that

contaminated toys with EV was 16 months old; the age when toys are not mouthed but the hands are. These data suggest that fingers that came from the child's mouth contaminated the toys, and not the child's mouth itself.

The findings of this study, therefore, suggest that the primary mechanism of EV transfer from symptomatic paediatric sources to healthy young adults is by the hand-to-hand route, particularly if source hand mouthing is directly involved.

Aerosol transmission did not occur with TraVerse, although there are no studies suggesting that respiratory EVs spread through small droplet aerosols.

11.4.2.3 Clinical Manifestations

The diverse phenotypes and tropisms observed in EVs are due to the great extent of genetic variability in the species.[786] Symptoms of EV infection in children can range from mild colds to the more severe acute flaccid paralysis characteristic of polio EV.[786] Primary viremia occurs after oral ingestion, which enables the virus to infect multiple tissues during the circulation of blood; often, this is where infection terminates. However, there are cases where the virus can be found in the CNS, causing neurological disturbances.[786,789,790] In adults, symptoms of EV infection commonly manifest as conjunctivitis.[48,127]

In this study, there were no signs or symptoms of EV infection that were particularly significant in the adult host population, and there was no evidence of risk of symptom development on exposure to symptomatic sources with the virus. In the paediatric sources, however, the one systemic sign that was of particular significance was febrile convulsion, with 19% (5/26) of sources presenting with the neurological phenotype. Francis et al. (2016) elucidated that febrile seizure was common in children aged from 6 months to 5 years and generally coincided with annual peak respiratory seasons.[791] In their observational study of febrile convulsions in children presenting to the ED in an Australian tertiary hospital, they further determined that, in 71% of cases, infection with at least one virus resulted in episodes of the condition. EV was identified in 20% of their cases, although no particular strain was predominant.[791]

Ninety per cent of EV infections are asymptomatic.[786,792] However, the viruses are nevertheless formidable in causing at least 20 syndromes of clinical importance that include encephalitis and meningitis, myocarditis and pericarditis, hepatitis, arthritis, and pancreatitis.[786,792] In this study, 29% of hosts had asymptomatic EV infection. However, their source pairs tested negative for the virus, which could be because they were prodromal or recovering from the infection, or because host EV infection in this study occurred in the community and not during the interaction.

11.4.2.4 Summary of EV Findings

To summarise the findings on EV transmission using the TraVerse method, EV large droplet transmission occurred but was statistically due to chance. On the other hand, transmission by self-inoculation was the most efficient mechanism of EV virus transfer followed by fomite contamination, because children were 40 times more likely to have the virus on their hands. The childhood behaviour of mouthing hands, toys, and objects was associated with the high titre of EV on the children's hands, which correlated with the mechanisms of virus transmission.

Clinically, febrile convulsions were statistically significant to paediatric EV infections, which correlates with the CNS tropism of the virus. On the other hand, a proportion of adults had asymptomatic infection; however, it is not clear whether this was because their source pairs were prodromal or recovering from an illness, or because of community-acquired infection.

11.4.3 Influenza Viruses (Flu)

There was no evidence of flu transmission via large droplet, hand contact, or fomite contamination, using TraVerse. This was of particular disappointment to the researcher, who expected many contributions to flu pandemic preparedness because, up until the TraVerse study, the clinic in infectious diseases was primarily an influenza research facility. There is much controversy on the primary mechanism of influenza transmission and the researcher hoped to lay it to rest, at some level. In theory, Flu transmission occurs by any of three mechanisms, either

individually or with each other and involves, bioaerosols, large droplets, and fomites.[27,793] The controversy arises from the supposition that Flu is primarily spread by large droplet, even though there is no tangible evidence for its support.[27,353,793] Meanwhile, incidences of aerosol transmission have been reported since the 1930s although very little importance attached to these findings.[27,793] The controversy exists because Flu transmission studies have only so far focused on the effects of interventions and not on the mechanisms of transmission and because there is inadequate research on the natural human-to-human transmission of disease.[353] Killingley et al. (2012) addressed the first underlying issue in their investigations using the human influenza challenge model[12] derived from Meschievitz et al.'s MFT model of RV transmission.[13] The TraVerse method addressed the second issue for the first time, which made the lack of tangible results even more regrettable. The controversy continues to the present. For example, although recent findings underscore aerosol involvement in Flu transmission,[331] including evidence of super-spreading aerosolised Flu during the 2009 pandemic,[794] the use of N-95 respirators are still not compulsory in pandemic preparedness plans; Australia is the only country to recommend the use of N95 masks in its preparedness plan, but then only for healthcare workers.[27]

Since TraVerse is the first study to investigate the natural transmission of Flu, it is conceivable that negative transmission findings by large droplet, hand contact, and fomite contamination are valid. However, two things make it difficult to prove, 1) the small numbers of flu-positive sources and hosts, and 2) the lack of bioaerosol data. The small numbers of flu infection in this study are due to the scaled down recruitment during the peak respiratory seasons. Research ethics and the University of Leicester, College of Medicine stipulated the non-involvement of medical students in the study two weeks before the exams. Although exams usually occurred after a winter break of at least 4 weeks, students generally took this opportunity to see family and to study. Recruitment could have continued until the last two weeks of winter break; however, it was the experience in this study that, for the most part, students who did not celebrate the holidays or did not go home to see family were also not too keen to become infected, even several weeks before the exams. Recruitment during the holidays would have probably

been counter-productive anyway; the hosts would more than likely have been symptomatic, these being the peak of respiratory infections; hence, they would have satisfied the exclusion criteria. Moreover, Flu activity in Leicester during the first two years of the study was mild to moderate, infecting mostly young adults and the elderly.[795,796] It was, nonetheless, fortuitous that recruitment scaled down during the winter as this provided rates of natural transmission during the inter-epidemic seasons, which removed the bias of increased diagnostic positivity during peak infections.

The lack of aerosol data also hampered elucidating the primary mechanisms of Flu transmission in this study. Since published data promoted that human-to-human transmission of Flu is primarily by large droplets, the original TraVerse protocol did not include bioaerosol sampling. However, recent MERS-CoV reports of possible aerosol transmission prompted the TraVerse team to look closely into studies involving the aerosol transmission of Flu. When the team decided to start collecting aerosol samples, the funds to purchase the equipment did not materialise until the study was only two interactions away from completion.

11.4.3.1 Clinical Manifestations

Four symptomatic sources shed FluA(H3) in the study; two had mono-infections of the virus. The systemic signs and respiratory symptoms common to both were fever, cough, DIB, hoarseness, nasal discharge, and sneeze, none of which were of statistical significance to Flu infection in the study. Meanwhile, there was one host who developed clinical illness without shedding a virus, after interacting with one of the paediatric sources infected with FluA(H3). Host respiratory symptoms included a dry cough that started on day 1 and lasted until day 5, and sneezing and a runny nose that started on day 4 post-infection and lasted until day 5. A headache was the systemic sign reported by the host from days 2 to 5 post-interaction. Similar to sources, there were also no clinical manifestations that were of statistical significance to FluA(H3) infections in adults.

Studies suggest that correlating flu burden in hospitalised individuals is difficult because symptoms associated with Flu infection are similar to those experienced

when infected with other viruses, all of which peak in winter.[797] Hence, 'influenza-like illness' is used during empirical diagnosis. However, a meta-analysis of flu volunteer challenge studies involving young adults by Carrat et al. (2008) determined that systemic signs that peaked sooner and resolved earlier than respiratory symptoms significantly indicate a flu infection.[798] This, therefore, suggests that even though the host did not shed FluA(H3), their signs, symptoms, and timing of illness indicate that they were, nonetheless, infected with the virus. Furthermore, that FluA(H3) transmitted from their symptomatic source pair, who shed the virus.

11.4.3.2 Summary of Flu Findings

To summarise the findings on Flu transmission using the TraVerse method, Flu transmission by large droplet, hand-to-hand contact, self-inoculation, and fomite did not occur, much to the regret of the researcher. This negative finding could be because source-host interactions were scaled down during winter, or because the primary mechanism for Flu transmission is by aerosol. Aerosol transmission was not properly evaluated with TraVerse because the original study protocol did not take this mechanism into consideration and, when a new protocol was drafted to include bioaerosol collection, the funds were lacking. Nevertheless, the absence of transmission strongly supports the aerosol transmission of the virus, and the scaled-down winter schedule removed the bias associated with peak infection rates.

However, there were hosts that had clinical signs of infection after interacting with sources that had mono-infections of FluA(H3). The signs, symptoms, and timing of host infection suggest that transmission of the virus occurred, even though shedding did not.

11.4.4 Human Coronaviruses (HCoV)

Little is known about the endemic strains of HCoV that include NL63, 229E, OC43, and HKU1 despite almost 30 years of research.[427] Regrettably, this study cannot

add anything novel to the literature, although it confirms what is already published. A total of 5% of symptomatic sources in TraVerse were infected with HCoV (table 16); four group 1 (NL63, 229E) infections occurred in the winter of 2013 and one group 2 (OC43, HKU1) occurred in winter 2015. The mean age of the sources at the time of infection was 16 months; their symptoms were generally mild although wheezing was particularly significant. Studies supporting these data suggest that HCoVs primarily infect children less than 2 years old.[407,426,427] Isaacs et al. (1983) determined an infection rate of 30% in children aged <6 years, using ELISA.[407] Talbot and colleagues ascertained an infection rate of 5% in those aged <2 years, and 4.2% in those 6 - 23 months old, using PCR.[426,427] Finally, Kaye et al. (1975) determined an HCoV-229E infection rate of 4% in those aged 5 to 19 years, using indirect hemagglutination tests for HCoV antibodies.[409] Furthermore, they determined a cyclical nature of infection, with 229E and OC43 alternating infections during the years.[409] Talbot et al. (2009) confirmed this sporadic detection of endemic strains of the virus after observing HCoV infections in children for 20 years.[427]

Some studies demonstrated that endemic HCoVs are extremely transmissible by the faecal-oral route, or by any of the other three mechanisms investigated with TraVerse.[407,409,427,435] Other studies suggest that children < 2 years of age are primarily infected with the endemic HCoV, while older adults or young adults with co-morbidities are more likely to become infected with SARS-CoV and MERS-CoV.[403,407,426,427,443-446,458,799,800]

11.4.4.1 Clinical Manifestations

Wheezing was the respiratory symptom discretely associated with paediatric HCoV infection in this study that was significant (table 22), with 40% of sources that reported episodes. This is just slightly higher than the 30% proportion that Isaacs et al. (1983)[407] determined for these viruses.

Two adult hosts that were PCR-negative, and that interacted with paediatric sources with mono-infections of HCoV1, did not shed the virus but reported signs and symptoms. None of the signs and symptoms reported was statistically

significant to HCoV1 infection in adults, in this study. Of particular interest was that one of these two hosts had ILI (≥6 symptom score) that lasted at least 3 days. Data on the source-host pair revealed that the host reported clinical illness on day 1 post-interaction, while the source had the highest viral load among all the sources that shed HCoV, with a PCR cycle threshold (Ct) was 25. By contrast, the host that did not have ILI developed symptoms on day 4 and the source pair had a lower viral load, with a Ct value of 35.

In using Ct values to measure viral load and then correlating viral load to hospital length of stay (LOS), Clark et al. (2016) ascertained that viral load correlates directly with clinical outcomes and LOS, regardless of virus type, patient age, health status, and duration of illness prior to hospitalisation.[801] Moreover, when associating the incubation period of SARS-CoV with disease severity, Virlogeux et al. (2015) ascertained that a shorter incubation period determined more severe disease pathology.[802] They suggested that a higher virus load indicates the speed of viral replication that results in severe disease, either because the host cannot mount an immune response fast enough or because the response is too aggressive.[802] Therefore, a severe outcome occurred in 50% of symptomatic hosts with presumed HCoV infection in this study. This host did not stay home, take antipyretic, or seek medical advice during the follow-up period, suggesting that HCoV infections in healthy adults are underestimated because they are left undiagnosed.

Although studies suggest that asymptomatic infection is common in HCoV infections, there was no evidence of it in this study. However, Dare et al. (2007) and Koetz et al. (2006) recommend caution when correlating HCoV infection with acute respiratory symptoms due to the lack of comparative data.[441,442]

11.4.4.2 Summary of HCoV Findings

To summarise the findings on HCoV transmission using the TraVerse method, similar to Flu, HCoV transmission by large droplet, hand-to-hand contact, self-inoculation, and fomite did not occur in this study. This negative finding may be because infections with epidemic strains of HCoV, such as MERS-CoV, are more

likely in adults, while infections with the endemic strains identified in this study occur more often in the children. However, although this study did not add anything to the little body of knowledge on HCoV transmission, it had nonetheless confirmed general findings published from other studies.

Clinically, wheeze significantly associated with HCoV1 infection in children in this study. Meanwhile, there were hosts that had clinical signs of infection after interacting with sources that had mono-infections of HCoV1, which suggests that transmission of the virus occurred, even though shedding did not. There were no signs and symptoms of significance to host infection with the virus; although, a severe disease of long duration occurred. Despite disease severity, the hosts did not stay home, take an antipyretic, or seek medical advice, which has implications for the transmission of the virus by this population.

11.4.5 Human Adenoviruses (HAdV)

11.4.5.1 Fomite Transmission

There was no evidence of either large droplet or hand-to-hand transmission of HAdVs in this study. However, fomite transfer was apparent, at a rate of 33% (table 19). HAdV infects the gastrointestinal tract of humans and is, therefore, transmitted through the faecal-oral route, similar to EVs.[524] However, most studies on HAdV transmission were conducted in ophthalmology clinics because of its importance in infectious conjunctivitis.[803-806] Detection of HAdVs on ophthalmologic equipment, therefore, established fomites as the primary route of spread in these settings.[805,807] Some argue, however, that improperly disinfected hands of healthcare workers transfer the virus to fomites.[804,807,808] [586,805]

In this study, the unwashed hands of paediatric sources better correlated to virus transfer on toys than mouthing; the fomite transmission rate decreased to 14% when only investigating mouthing and then to 7% when considering both mouthing and hand contact. Furthermore, the paediatric source that contaminated the toys was 29 months old. Based on the mouthing preferences of children, the

child more than likely mouthed the fingers first and then transferred the virus to the toys from their hands. Therefore, it is suggested here that the primary mechanism of natural HAdV transmission is by fomite, the virus transfers to fomites through unwashed hands, and transmission occurs within 20 minutes of contact with hands.

11.4.5.2 Clinical Manifestations

In this study, HAdV infection in 14 paediatric sources significantly associated with fever (table 22). However, 86% (12/14) of the sources had co-infections with RV (57%), EV (21%), and FluA(H3), HCoV, hMPV, PF3, RSVA, and RSVB (each at 7%). Furthermore, 75% (9/12) of those with co-infections were aged <24 months. These findings are not novel, but comparable to other studies in children infected with HAdV. For example, Putto and Meurman's (1986) research into paediatric respiratory diseases associated with fever established that 68% of those with fever (≥39˚C) shed HAdV.[809] Lee et al. (2016) studied the clinical impact of mixed infections in Korean children admitted to hospital with HAdV, using PCR.[759] In their study, patients with double and triple infections presented with fever that had a median duration of 5 days. When they compared groups with HAdV mono-infection and co-infection, they elucidated that there were no significant differences between the two groups in terms of fever duration and rates of hospitalisation. Moreover, they observed that a higher proportion (71%) of children aged <24 months had co-infections and were more likely to have underlying conditions. The viruses often associated with HAdV co-infection in their study were, RV (42%), RSV (19%), HCoV (17%), FluB (3%), and hMPV (3%).[759]

The adult hosts paired with the two paediatric sources who had mono-infections of HAdV did not shed the virus but, nonetheless, developed respiratory symptoms, suggesting transmission of infection occurred (table 19). However, none of the hosts had a fever, ILI, or symptom duration ≥3 days. Therefore, although clinical infection of healthy young adults occurred after exposure to symptomatic sources infected with HAdV, severe infection was not observed in this study.

11.4.5.3 Summary of HAdV Findings

To summarise the findings on HAdV transmission using the TraVerse method, HAdV transmission by large droplet, hand-to-hand contact, and self-inoculation, did not occur in this study. However, fomite transmission occurred in one-third of interactions where paediatric sources had HAdV infections. Fomite transfer occurred within 20 minutes of infected children touching toys.

Most studies on HAdV were done in ophthalmology clinics because the virus causes infectious conjunctivitis in these settings. Therefore, most of these studies attributed the spread of the virus with fomites. This study contributed to knowledge on HAdV transmission with its finding that the primary mechanism of HAdV transmission in a natural, non-ophthalmologic setting is also by fomite. However, caution is needed in interpreting this finding because the aerosol transmission of viruses was not evaluated in this study.

Although HAdV is an enteric virus like EV, mouthing was not involved as much as hand contact in the transfer of virus on toys, likely because the children infected with the virus in this study were at an age where mouthing of fingers is more common than placing toys in their mouth. This finding further agrees with the theory that the unwashed hands of staff in ophthalmologic clinics transfer HAdV onto equipment and surfaces.

Clinically, fever was statistically associated with a HAdV infection in children in this study. A large proportion of these children with a fever were also infected with other viruses and were aged <24 months, similar to other studies. Two hosts reported clinical signs of infection after interacting with sources that had mono-infections of HAdV; however, fever was not present, and the infection in adults caused mild respiratory symptoms.

11.4.6 Respiratory Syncytial Viruses (RSV)

11.4.6.1 Large Droplet Transmission

Nosocomial RSV infections place a substantial burden on paediatric healthcare settings during epidemic seasons and often result in ward restrictions to interrupt the cycle of transmission.[633,672] There are several protocols implemented for the prevention and control of RSV transmission, each with varying levels of success,[633,645,658,672] presumably because the mechanisms of virus transfer are not suitably delineated. Studies suggest that large droplets and self-inoculation with hands that had contact with fomite are the primary mechanisms of RSV transmission.[26,645] There is also some evidence that aerosol transmission occurs.[810,811] However, the contributions of each mechanism to the transfer of the virus are not clearly defined. Therefore, the rates of RSV transmission established using TraVerse are novel findings.

In this study, large droplet transmission of RSVB occurred at a rate of 5% (table 19). Healthy young adults do not generally shed large amounts of viruses during infection; hence, it is likely that host shedding occurred at levels too low to for PCR detection, which accounts for the low transmission rate. Furthermore, there were three hosts that shed RSVA and RSVB, but whose source pairs did not shed either virus. At the time of sample collection, RSV in the host population was either circulating at very low levels or not at all.[795,796] Therefore, it is conceivable that these infections were, in fact, from their source pairs that shed at titres too low for PCR detection, such as when they are on their way to recovery. RSVA large droplet transmission was not observed in this study.

11.4.6.2 Hand-to-Hand Transmission

RSVA was detected on the hands of 43% (3/7) of the sources that shed the virus in this study (table 16). Furthermore, there was an extremely statistically significant risk that 43% of sources shedding RSVA would contaminate their hands with the virus, which is 58 times more likely to occur than if the sources were not shedding RSVA (table 26). However, none of their host pairs had the virus on their hands or

face, suggesting that RSVA is not easily transmissible by the hand-to-hand or the self-inoculation routes, in this population.

By comparison, RSVB was detected on the hands of 48% (10/21) of the sources that shed the virus (table 16). The risk of the sources shedding RSVB and contaminating their hands with the virus was extremely statistically significant at 45%, which is 24 times more likely to occur than if the source did not shed the virus (table 26). Therefore, it is less likely for RSVB hand transfer to occur in paediatric sources than it is for RSVA. Regardless, 18% (2/11) of sources transmitted RSVB to their host pairs; 50% of these hosts subsequently inoculated their own face (table 19). Furthermore, the hand-to-hand transmission of RSVB was 23 times more likely to occur than if the source pairs did not have the virus on their hands (table 27). Meanwhile, self-inoculation by hosts that had RSVB on their hands was 73 times more likely to occur than if they did not have the virus on their hands (table 27). Therefore, these findings suggest that the primary mechanism for RSVB transmission is by the hand transfer of the virus and not by droplet infection.

Interestingly, 77% (10/13) of sources that had either RSVA or RSVB on their hands were at the age where mouthing is indicated. Although, this could just be because RSV infection is most common at the ages when mouthing also occurs.

11.4.6.3 Fomite Transmission

Fomite transmission of RSV was not observed in this study. This is contrary to studies suggesting that RSV infection occurs from self-inoculation after touching objects and surfaces contaminated with large droplets.[26,32,612,644,645] These studies presented findings that include, RSV survival in the environment for 6 hours, on rubber gloves for 1.5 hours, and on cloth materials for 45 minutes.[617] However, because these studies elucidated the effectiveness of intervention methods, it is conceivable that they overlooked the importance of the hands of paediatric sources and adult hosts in the transmission of the virus. For example, in Hall et al.'s (1981) research on the various routes of RSV infection, adult volunteers were inoculated with different titres of virus in the nose, eyes, and mouth.[812] This

suggests that there was already a presumption that infection can only occur by any of these three routes. Furthermore, the role of children in the infection adults through these routes was not considered. Studies also determined that RSV survival on human skin does not exceed 20 minutes;[617] yet importance was placed on this finding only inasmuch as how hand washing and surface decontamination could prevent hand infection, and not that hand-to-hand transfer is a possible mechanism of transmission, let alone that it is the primary mode of RSV transmission.

The aerosol transmission of RSV was not established using TraVerse. However, there is evidence to suggest that this mechanism of transmission is possible. This was demonstrated in Kulkarni et al.'s (2016) research on infants with bronchiolitis, where large numbers of infectious, aerosolised RSV particles were detected. They concluded that the aerosolised particles remained suspended in the air long enough for nosocomial infection to occur.[811] Grayson et al. (2017) collected aerosol samples in paediatric outpatient clinics during peak RSV infections and established that RSVs were present in aerosols in these settings. However, they were not as convinced as Kulkarni that transmission by this route is particularly efficient.[810]

Effective infection control methods benefit greatly from knowledge of possible sources of contamination. Several combinations of barriers to infections through large droplets, fomites, and aerosols are implemented during annual RSV epidemics, with varying degrees of success. It is suggested that the best method combines staff hand washing with cohorting while using gloves and gowns likely enhance transmission.[633] It was established in this study that hand-to-hand transmission occurred as soon as 10 minutes of close interaction between source and host, suggesting that RSV is a highly opportunistic pathogen, considering the virus only survives on human skin for 20 minutes. Therefore, this begs the question of whether hand washing is necessary if the virus was going to spontaneously die off anyway. It is more prudent that interventions involve preventing self-inoculation after hand transfer, such as with masks and facial wipes.

The cohorting of patients could be beneficial in the aerosol transmission of RSV. However, studies suggest that the practice is not relevant. Specifically, Thorburn et al. (2004) observed that the spread of RSV stopped despite eschewing isolation units in favour of treating paediatric inpatients in open wards.[813] Meanwhile, Grayson et al. (2017) asserted that aerosols are inefficient methods of spreading RSV infection.[810] Moreover, cohorting during peak RSV epidemics is impractical and logistically challenging.

The use of gloves and gowns were implemented when it was determined that direct contact with secretions was relevant in RSV transmission.[812] However, reports mentioned in earlier paragraphs, suggesting that RSV survives longer on gloves and cloth materials than fingers,[617] may have implications for further virus spread. Fomite transmission was not established in this study, despite the high titres of virus on hands. Therefore, it is suggested here that, although gloves and gowns are not necessary for RSV infection control, neither do their use pose a risk, because infection through these fomites is unlikely.

11.4.6.4 Nosocomial Infections with RSVA and RSVB

Notable in this study were the viral loads observed for both RSVA and RSVB, in both sources and hosts, which were the highest in all the viruses evaluated using TraVerse (table 18). Studies suggest that RSV transmission is particularly relevant in nosocomial infections because children shed the virus at high titres for prolonged periods.[644] However, this was not observed in this study because, although source viral loads were high, they did not statistically show correlation with either RSVA or RSVB transmission to the adult host. Moreover, data on source duration of shedding were not captured in this study.

In adult hosts, RSVA infection had a short incubation period of 24 hours, and duration of shedding was seven days. Meanwhile, RSVB had a longer incubation period between two and six days, with a median shedding duration of two days (table 20). These findings in the hosts are supported by a study by Hall et al. (2001), which reported a mean RSVB shedding duration of 3.9 days.[651] Meanwhile, a systematic review by Lessler et al. (2009) estimated an incubation range of 3-7

days,[751] which also supports the RSVB findings in this study. However, Lessler et al.'s (2009) values were a composite of all RSVs in all age groups. Using TraVerse, concrete differences were observed in RSVA and RSVB transmission rates and risks for infection in both children and adults; hence, it was suggested here that composite reporting is not the best way to present RSV findings. Instead, RSV results should be interpreted according to the virus type.

Nosocomial RSV infection is most relevant in paediatrics wards, where 45% of younger children that have had longer hospital stays are infected.[633,658] Given that, in this study, RSVA was more likely than RSVB to be found on the hands of children that are shedding the virus, RSVA had shorter incubation periods and longer shedding durations than RSVB, and RSVA was not transmitted to adults hosts, then perhaps RSVA is relevant in the nosocomial infection of children by other children that are infected with the virus. Meanwhile, the nosocomial infection of healthcare workers may be due to the infection of adults by children with the RSVB on their hands. These findings should be interpreted with caution, however, because child-to-child transmission was not evaluated in this study. It is further suggested here, therefore, that RSV transmission should be distinguished between children and adults and that studies relating to the natural transmission of RSV from one child to another be further pursued.

11.4.6.5 Clinical Manifestations

Using Traverse, this study determined that RSVA infection in paediatric sources statistically correlated to their developing systemic signs, but not respiratory symptoms (table 22). On the other hand, RSVB infections were not significantly associated with respiratory symptoms or systemic signs, suggesting that RSVA infections may be more severe than RSVB in children. These findings further indicate that RSVA and RSVB have different disease pathology; hence, reporting findings according to subtype would provide a more accurate representation of each.

Studies on paediatric RSV note the importance of LRTI in this population, particularly bronchiolitis and viral pneumonia.[658] However, there are no data

collected on either of these conditions in the study. Furthermore, only 4 out of 27 RSV-positive sources had a working diagnosis of bronchiolitis on admission. This is likely because 21 out of 27 had RSVB, which had a milder disease presentation in sources in this study. This is supported by Virlogeux et al.'s (2015) premise, mentioned in an earlier chapter, that short incubation periods and high viral titres correlated with severe disease.[802]

By contrast, RSVB had a more severe disease presentation in healthy young adults in this study, with both respiratory symptoms and systemic signs significantly correlating with subtype (table 24). Severe RSV disease in healthy, working adults is not very much appreciated, mostly because their symptoms are thought to be generally mild and self-limiting; their risk for severe disease is, therefore, negligible.[32] This misconception is reinforced by the fact that young adults do not tend to seek medical attention, and so are not diagnosed. Moreover, because there are no over-the-counter medications specific for RSV symptoms, disease cannot be correlated with adult health-related spending.[32] However, research involving nosocomial RSV suggests that disease is severe enough in healthy young adults working with children, to account for the number of missed days at work.[32,645,814] In prospectively studying nosocomial infection, Hall et al. (1978)[49] observed healthcare workers for two months. They determined that, although most adult RSV illness was confined to the upper respiratory tract, still an average of six working days were missed by 80% of individuals.[49] In another study, Hall et al. (2001) determined that 84% of previously healthy working adults developed symptoms due to RSV infection. Although most of these infected adults worked at hospital or medical university, contact with paediatric patients did not occur in more than 50%. Meanwhile, asymptomatic infection was rare and observed only in adults who have worked in paediatric wards for many years.[651] This particular study supports a few of the findings in TraVerse including, the statistically significant risk of hosts developing clinical illness after RSVB transmission by sources (table 27), the lack of direct correlation of host RSVB infection with source shedding and viral load, and the absence of asymptomatic infection in hosts.

11.4.6.6 Summary of RSV Findings

To summarise the findings on RV transmission using the TraVerse method, RSV large droplet transmission occurred at a low rate of 5%, likely because the sources were prodromal or recovering from illness, or the hosts were shedding at low levels not detected by PCR.

Meanwhile, hand-to-hand transmission of RSV was the primary mechanism of transmission, although only RSVB transmitted, and not RSVA. The transmission of RSVB to adults was 23 times more likely to occur if the children had the virus on their hands than if they did not. Half of the hosts to whose hands RSVB transferred touched their faces, which had implications for clinical illness in this study.

Virus shedding of RSVA and RSVB was the highest observed in this study, with both children and adults shedding the viruses at titres up to two logs more than Flu. There were distinct differences in the incubation periods and shedding durations between RSVA and RSVB; therefore, a PCR assay that subtypes viruses in recommended for RSVA diagnosis.

Fomite transmission did not occur in this study, which contradicts studies that suggest RSV transmission occurs by the hand-to-fomite-to-hand route. Instead, this study established that RSV transmission is primarily by the hand-to-hand-to-face route, which has implications for nosocomial infection.

Nosocomial infections are highly relevant in RSV infections and are highest in children than in adult healthcare workers. NI is suggested to correlate to high virus titres and long durations of shedding, both of which were observed with RSVA but not RSVB. Since RSVA was not transmitted to adults, it was theorised that RSVA is only transmitted from a child to another child and not from a child to an adult. However, because child-to-child transmission was not evaluated using TraVerse, more research needs to be done.

Clinically, RSVA infection in children was associated with severe systemic signs, while RSVB infections correlated with a temperature≥37.8°C. Bronchiolitis and pneumonia commonly occur in RSV infections in this population but were not

observed in this study, likely because most of the paediatric infections were with RSVB. By contrast, RSVB resulted in severe disease in adults in this study, which was characterised by systemic signs that included myalgia. Meanwhile, RSV as a group caused upper respiratory symptoms such as sore throats, and systemic signs that included malaise. Therefore, the suggestion that RSV disease in adults is generally mild and self-limiting is a misconception, likely because this population does not seek medical attention and so are not diagnosed. Because the adults are not diagnosed, the sales of over-the-counter medications cannot easily be associated with the burden of RSVB in this population.

11.4.7 Human Metapneumovirus (hMPV)

Human metapneumovirus is one of the common cold viruses recently identified using PCR. Although it was only discovered in 2001, molecular evolutionary studies determined that hMPV has infected humans for at least 50 years.[2,29,611,688] However, even 16 years since its discovery, little is known about the virus, presumably, because it is not included in many standard laboratory diagnostic screenings.[2,19] Therefore, this is the first study to elucidate the human-to-human transmission of hMPV and to do so in natural infection. However, data from this study should be interpreted with caution.

In this study, seven paediatric sources shed hMPV; three had mono-infections, and four were infected with other viruses. However, hMPV transmission did not occur by any of the mechanisms evaluated. Other studies established that hMPV behaves much like RSV;[815] however, although RSV droplet and hand contact transmission were observed using TraVerse, neither was evident with hMPV.

Notable were the high virus titres that the paediatric sources shed. The least amount of virus shed had a Ct value of 29, which was the Ct of the severe case of adult HCoV infection in this study. Meanwhile, the highest amount of virus shed; hence, the most severe symptoms in sources with hMPV, had a Ct of 13. Therefore, hMPV infection in the paediatric population, in this study, resulted in severe clinical illness.

In healthy young adult hosts, hMPV infection did not occur. However, two adults whose source pairs had mono-infections of hMPV reported symptoms. Given that the sources shed hMPV at high titres and that the virus was not circulating in the host population, suggest that hMPV transmission occurred, and at a considerable rate (table 19). The mechanism by which host infection transpired was not established using TraVerse. Although given that small droplet aerosol was the only mechanism not properly investigated, it is feasible that aerosol transmission was the primary mode of hMPV transfer, in this study.

11.4.7.1 Clinical Manifestations

Human metapneumovirus belongs to the same family of viruses as RSV. Hence, most studies find that the virus causes similar symptoms as RSV bronchiolitis with the exception that hMPV is also associated with URTI, LRTI, and diarrhoea accompanied by fever.[688,736,816-820] A broad hMPV prevalence range of 3.3 to 19 is reported, depending on whether hospitalised children with underlying disease were included or not.[821] It is established that hMPV is second only to RSV in incidences of acute bronchiolitis in paediatric inpatients. Furthermore, the virus can cause infections on its own such that, with a co-infection, the risk for mechanical ventilation increases.[821]

In this study, a prevalence of 7% was observed in paediatric hMPV infections, similar to RSVA, and is well within the range reported in other studies. Single infections were observed in 43% (3/7), while co-infections occurred with EV, HAdV, HCoV, and RV. Fever was observed in 3 (43%) of the sources; one (14%) of whom also had diarrhoea. A novel finding in this study was that six (86%) of the sources with hMPV infections were females aged between 4 months and 39 months; this finding was significant (p=0.016), and the odds of it occurring were 10 times greater than if the children were male. Therefore, compared to recent studies on children hospitalised with hMPV, [815,822] TraVerse findings are concordant, except for the preponderance of the disease in female children. However, more data are needed to establish the clinical significance of gender in paediatric hMPV infections.

Studies on hMPV in healthy young adults are more limited than those involving children. Nevertheless, it is suggested that the burden of hMPV infection in adults is substantial. Widmer et al. (2014) determined a hospitalisation rate of 2.6% with a proportion of ED admissions that was six times higher.[823] These rates increased with age, as observed in recent cases in the elderly that had a fatality rate of 50%.[690]

Although the mechanism of hMPV transmission was not established in this study, two out of three adults that interacted with sources that had mono-infections of the virus, developed illness nonetheless. There were no signs or symptoms that statistically correlated with adult infection, although both hosts reported a dry cough and nasal congestion. Both hosts also had illness duration ≥3 days, which are supported by findings that illness in young adults can last up to ten days.[691] Therefore, although hMPV transmission was only presumed in this study because adult PCR test results were negative, the number of healthy hosts that became ill for several days after interacting with sources infected with hMPV, was nonetheless substantial (67%). These findings are concordant with the limited studies in this population, which provide further evidence that hMPV causes severe disease in healthy adults that have implications for the number of days lost at work.

11.4.7.2 Summary of hMPV Findings

To summarise the findings on hMPV transmission using the TraVerse method, transmission by large droplet, hand-to-hand contact, self-inoculation, and fomite did not occur with hMPV in this study. Despite a prevalence of infection in children that equalled that of RSVA, this negative finding in the study could be because aerosol is the primary mechanism of transmission of the virus.

Clinically, hMPV infections in children in this study is characterised by mono-infections in 43%, and a fever that is often accompanied by diarrhoea, which is similar to findings in other studies. However, a novel finding in this study was that females were statistically more susceptible to hMPV, at a rate of 86%, and that they are ten times more likely to be infected with the virus than are males.

In adults, 67% became ill without shedding a virus, after interacting with source pairs that had mono-infections of hMPV. Disease in adults was mild, and no particular signs or symptoms were of significance, which contradicts other studies. However, the long duration of adult clinical illness from hMPV infections is congruent with other studies. Therefore, there is still very little known about hMPV infections even though it was discovered 16 years ago and has infected humans for 50 years. This study, using TraVerse, adds a little more to the body of knowledge; however, including hMPV in standard laboratory diagnosis is the best way to know more about the virus.

11.4.8 Parainfluenza Viruses (PIV)

Using TraVerse, natural PIV transmission by large droplet, hand contact, and fomite was not observed. This finding is contradictory to other studies that suggest PIV transmission occurs through large droplet, contact with unwashed hands, or aerosols [694,701,805] because infections with this virus cause community epidemics and nosocomial infections.[805]

However, despite not having elucidated PIV transmission rates, this study established that clinical manifestations of infection in children differ according to the virus type, another indication that a PCR method that identifies virus infection by subtype is clinically relevant. Furthermore, this study determined that hosts who did not shed PIV nonetheless became ill after interacting with their source pairs who had mono-infections of the virus, at rates of 100% for PIV1 and 75% for PIV3 (table 19). Therefore, there is clinical evidence that PIV1 and PIV3 transmission occurred using the TraVerse method and, because aerosol transmission was not properly evaluated in this study, it is conceivable that this was the mechanism by which virus transmission occurred.

11.4.8.1 Clinical Manifestations

Parainfluenza viruses are relevant in epidemic infections because they tend to spread quickly in the community, diseases result in considerable hospital

admissions, and nosocomial infections in neonates, the chronically ill and bone marrow transplant (BMT) patients are critical and result in the closure of wards for a protracted period.[805,824] PIV3 is particularly hazardous in BMT units, where they cause mortality in 50% of infections.[808,825] Because it is difficult to confidently identify the mechanisms of transmission in these settings, current infection control practices have not proven to be appropriate or consistently effective.[808,825] Infection control is particularly problematic because patients tend to shed PIV for long periods; sometimes for up to 4 months.[718,808] PIV epidemics generally occur between May and September in temperate climates, a long period that suggests the occurrence of multiple introductions into the community and the wards. Full-scale infection control practices are strictly imposed in the wards where nosocomial infections are most critical;[808] these include using gowns, single-use gloves, patient and carer cohorting, and proper hand washing. More importantly, symptomatic staffs are strongly advised to stay home from work; superiors are particularly vigilant about even minor sniffles that could later prove harmful to the patients.[808,826]

In this study, 9% of paediatric infections were due to PIV3, which were prevalent during spring. This prevalence was higher than that seen with other members of *paramyxoviridae*, RSVA and hMPV, which both measured 7%. By contrast, PIV1 was identified in 2% of the paediatric sources, the second lowest prevalence measured in this study, after HCoV2. PIV2 and PIV4 were not shed by the sources, which is supported by studies suggesting that PIV2 circulates in irregular patterns[694,701,702,711] and that PIV4 is rare but, when present, causes significantly severe clinical conditions.[701,713]

Clinical manifestations were more severe in PIV3 infections in paediatric sources in this study and included tachypnoea, lung crackles, and wheeze. Fever was a very strong indicator of infection with both PIV1 and PIV3 (table 22). Chughtai et al. (2017) promoted the consideration of co-infections when clinically assessing children that present with fever, because fever is a likely indicator of co-infections, and co-infections result in more severe clinical manifestations.[827] However, the difference in symptom severities between PIV1 and PIV3 cannot be correlated with co-infections in this study, with each type having co-infection rates of 50%.

Chughtai et al. (2017) determined a rate of 8.3% for fever in adults with PIV infections and a correlation of co-infection with adult fever. They also determined fever to be appreciatively higher among healthcare workers in their study.[827] None of the adult hosts that interacted with the sources infected with PIV reported a fever; therefore, results in this study cannot be compared to Chunghai et al.'s (2017). A fever is an inflammatory response. Therefore, if taking Virlogeux et al.'s (2015) premise that high viral loads are associated with severe disease because the host immune response is either too weak or too aggressive, then the reason why hosts in this study did not develop a fever or shed viruses despite clinical signs of disease could be because they had a robust immune system that had the appropriate response to infections. A serological analysis is needed to challenge this premise.

Despite the absence of a fever, however, the host whose source pair had a mono-infection of PIV1 reported systemic signs, including fatigue, malaise, and myalgia. None of these signs were statistically significant to PIV1 infection in adults, although they have implications for missed days at work. On the other hand, hosts whose source pairs had mono-infections of PIV3 reported only upper respiratory symptoms, none of which were statistically significant to PIV3 infection in adults. Therefore, although the exact mechanisms of PIV1 and PIV3 transmission were not established using TraVerse, clinical infection in adults after interacting with children infected with the viruses nevertheless suggests that disease transmission occurred.

Since aerosol transmission was not properly evaluated, it is suggested here that it could be the primary mechanism for PIV transmission. Furthermore, even though none of the signs and symptoms reported by the hosts correlated with PIV type, the results suggest that clinical manifestations differ according to the virus type in both children. Finally, it was established that PIV subtypes differ in clinical manifestations in children and young adults, which may have implications in the clinical management of the disease. Therefore, a PCR method that identifies PIV infection by subtype is clinically relevant.

11.4.8.2 Summary of PIV Findings

To summarise the findings on PIV transmission using the TraVerse method, transmission by large droplet, hand-to-hand contact, self-inoculation, and fomite did not occur with hMPV in this study. Despite a prevalence of PIV3 infection in children that was higher than other paramyxoviruses, this negative finding in the study could be because aerosol is the primary mechanism of transmission of the virus.

Clinically, PIV1 and PIV3 infections in this study cause severe disease in children that resulted in LRTI. Fever is significant to severe infections in children with either PIV1 or PIV3, which other studies suggest is due to co-infections with other viruses. However, the correlation between paediatric fever and co-infections cannot be assessed in this study because there were equal rates of mono-infections and co-infections in children with PIV.

In adults, 75% became ill without shedding a virus, after interacting with source pairs that had mono-infections of PIV3; the rate increased to 100 when the source pairs had PIV1 infections. However, fever and severe disease were consistently absent in the adults in this study. The reason provided here was that adults had a robust immune system that was able to mount a response to infections that was neither too weak nor too strong, which resulted in mild, self-limiting symptoms of short duration.

11.5 Difficulties Encountered

11.5.1 Source Recruitment

This project had the most number of research participants of all transmission studies that are known. There were 154 paediatric patients recruited and 191 healthy young adult subjects on reserve. These numbers give one the impression that the pool of participants was large, or that obtaining consent was a relatively easy exercise, given the nature of the project – young medical students playing with sick children, in the hospital. It was relatively straightforward recruiting

potential hosts who came to the clinic in droves and banged on research doors, begging to be consented into the study. The researcher assumed that it was their genuine interest in the advancement of medicine and research that brought about this positive response and that the monetary incentive was just incidental to their interest. Hence, recruiting the hosts did not pose that much of a difficulty although, they caused complications in other aspects of the study that is discussed in the next section.

The recruiting experience was not the same with the paediatric sources, and this was where the most struggles were experienced in this study. There were times when the researcher would go for days without obtaining parental consent, which was demoralizing. On slow days, not being able to recruit patients was tough to bear because not many patients were eligible to participate, and desperation set in. On days when the ward beds were at full capacity, there were too many from whom to choose, but the need of the medical team to rapidly assess and discharge patients took precedence over any research being done; on these occasions, the researcher got in the way of medicine in practice. And then there were the 'sweet spot days,' when the wards had neither too little nor too many eligible patients from which a researcher can choose. Those were the days when a negative parental response was most challenging to withstand because it meant one was doing a very bad job of obtaining consent.

Lebet et al. (2013) provided nurses' insights on best practices in asking for parental permission in clinical trials involving children,[828] while Sammons et al. (2007) established the motivations of British parents in enrolling their children to participate in ethically approved clinical research.[829] They determined that parents were more likely to provide consent if, they were not feeling pressured into it, they perceive that the study is important in gaining medical knowledge for the future, the child benefits are clear while the risks are low, and the study does not interfere with normal clinical procedures. By contrast, parents who refused consent cited reasons such as, rushing through the information and consent process, the perception that their child's care will be compromised or delayed, anxiety regarding the child's illness, chronic illness in any of their children, a higher level of education, and the consent form clause on who eventually gets

access to the records.[828,829] However, it is the researcher's opinion that the procedures followed in obtaining consent in this study were technically on-point. The information sheets and consent forms were ethically approved and easy to read and understand, the researcher had knowledge of all aspects of the study, received training in consenting parents and young adults and got better with experience, and had a good rapport with the junior doctors and consultants working in ED and in the wards.

Lebet et al. (2013) further impressed upon the need for teaming up with the clinical staff, particularly the nurses. They established that nurses are trusted more than anyone in the clinical team such that, their viewpoint held enough sway for either a positive or negative consent outcome.[828] Furthermore, involving nursing staff who personally care for the potential recruits is invaluable to the researcher because, they know the patients and the family dynamics involved, can advise on the best times to approach a parent to discuss the study, and can provide credibility to the research team, which the parents then take on board when deciding to agree to the study.[828]

In hindsight, the pivotal point in recruiting paediatric participants into this study could have been when the researcher established rapport with the ward sister and her deputy. There was no monumental event, just a series of little events that somehow eventually made the researcher part of the ward team, rather than the invading outsider. For example, at times when the ward was incredibly busy that treatment rooms used for the interactions in this study were not available, the researcher helped facilitate patient movement by supporting the nursing staff in such things as: handing out baby formula to the parents to keep track of their child's food intake, or providing clean diapers and weighing wet ones to record urine output; both of which needed to be fulfilled before the patient can be discharged. The moment a child tugged on the researcher's clothes to hand over her little brother's urine and stool sample because her mother said so was, indeed, a memorable day. More importantly, when evaluating bedside testing for respiratory viruses was being evaluated for immediate implementation, the researcher volunteered to train the nursing and support staff in performing the test and created a form for reporting critical information that can be analysed by

the team assessing the tests. In return, the researcher was not treated as unkindly when using treatment rooms for interactions, even on extremely busy days. The researcher also received proper advice on who was eligible to take part, who in ED were making their way up to the wards, how many were expected to come in at nights, on the weekends, and during holidays, and what times were best to recruit. Eventually, the researcher was offered toasted bread for breakfast and tea at any time of day, like any other staff. Therefore, on this occasion, the researcher integrated into the wards for the mutual benefit of research and medicine. Integrating was unintentional but, it became a necessity since almost all day was spent in the wards anyway; hence, it may not be for everyone. However, the give-and-take nature of the interaction between researcher and ward staff is essential and should not be taken lightly.

11.5.2 Host Logistics

This research was always going to be a logistical nightmare because, not only do paediatric sources need to meet inclusion and exclusion criteria, healthy young adult hosts must as well, and then they both have to be available at the same time for the interaction to occur. Aside from that, recruiting hosts must only be done until two weeks before an exam, host follow-up visits must be timed so that they do not impede recruitment of the next source-host paired interaction, and these visits must be made outside lecture hours so that the hosts are not late for classes. Therefore, a high level of organization was necessary for the project to go as smoothly as a well-oiled machine. However, no matter how organized one is, there will always be a spoke in the wheel.

The most difficulty encountered with the hosts was the dynamism of their schedules such that, even though the researcher was aware of their availabilities two weeks beforehand, sudden changes never failed to surface. Changes were usually due to additional lectures that they elect to attend, or anxiety over exams that were several months away. These schedule changes tended to coincide with the times when sources willing and eligible to interact with hosts were abundant. These changes impeded the study because they prevented or delayed commencing the source-host interactions, which was a particular nuisance because they wasted

the two to three hours it normally took to consent a child into the study. Most interactions cannot be delayed because the children were already discharged by the time the host finally turned up at the clinic. After a while, the researcher noticed that hosts in the same year of studies had relatively similar days and times of availabilities. Since participating in the study had a reasonably substantial financial incentive for hosts, the researcher operated on a system of contacting hosts according to the order in which they submitted their fortnightly schedules. However, when it was evident that a schedule change has occurred, then every available host in the year/s affected by the change were bypassed, in favour of those in other years. This had negative implications for recruitment in that it decreased the pool of adults on the day's list. However, it also prevented wasting the time of the researcher, the medical staff, and the parents, and the disappointment of the source in not being able to play.

Another nuisance relates to the follow-up visits being scheduled according to the hosts' free time. Although this was very well tolerated and accepted by the researcher as necessary, there were instances when it inconvenienced the researcher. Such occasions included arranging follow-up swabs after visits to the gym at 6AM, after weekend family visits at 11PM, and during weekends and holidays; this being the norm and not an irregularity. These occasions cannot be helped and may just be best considered the occupational hazards of prospective clinical research.

Not long after the first year of study, TraVerse became established as the fun study that pays UoL students to participate. This made it so that, when the hosts were not able to interact with a source, they let their friends know, who then contacted the research team directly to volunteer. This was generally allowed, as long as there was no one else in the queue of hosts to be contacted, and the alternate hosts were already on the reserve list. Since the interactions were time-critical to the sources getting discharged, hosts were asked to arrive at the clinic for consent, swabbing and blood collection within 30 minutes of communications with the researcher. This was considered reasonable since the university was only a 10 to 15-minute walk downhill to the hospital clinic. However, because hosts did not want to lose their place in the queue for interactions that day, they would send a

message that they were on their way to the clinic, but not turn up until after at least an hour has passed. More often than not, the hosts were able interact with sources, but this placed time pressure on everyone involved, including the parents and the sources. Furthermore, these interactions tended to be of short duration; either the sources were tired because they had to wait, or their parents were eager to leave because their other children were waiting at school or at home. Therefore, a time limit was set up to reduce these incidences as much as possible; the hosts were advised that failure to turn up at the clinic within the allotted 30 minutes forfeited their participation and passed on the opportunity to the next host in the queue.

Finally, although there were no shortages of host volunteers in the study, with equal proportions of males and females, there was, nonetheless, a disparity in the ethnicity of the participants. In the years of study, there were approximately 1200 young adults matriculating at the UoL Medical School annually (data provided by UoL); the ethnic proportions were, 54% White, 24% Asian, and 22% others. However, the hosts that participated in the interactions were 70% White. Studies suggest that involving minorities (including women) in research is a difficulty in many studies.[830-832] Minorities are important in research, to make sure that findings apply to the general population, to promote healthcare equality, and to uphold ethical research standards.[830-832] It is suggested that minorities do not participate in research for personal, historical, and practical reasons; that is, a personal distrust of studies, a history of studies-gone-awry that involved minorities, and the difficulties in getting to research centres.[830,832] Suggestions for overcoming these barriers include monetary incentives, flexibility in participation, different mechanisms of contact, including social media and use of other languages, and emphasizing the benefits of participation.[831,832] The researcher believes that these suggestions were utilized in TraVerse, except that English was the only language used, as required by the study research ethics committee. However, if the reason for minority students' non-participation in the study was because of ingrained research distrust, it is doubtful that there was much else to be done to encourage participation. It is, however, hoped that TraVerse was a positive

experience for the volunteers, so that it may encourage research participation in the future.

11.5.3 Funding

The difficulty in procuring a bioaerosol sampler was already alluded to in previous sections, and its consequence was apparent in the absence of usable data on aerosol transmission in this study. The other difficulty encountered, due to lack of funds, was in the whole genome sequencing (WGS) of RV samples: there was a limited number of laboratories that were capable of the WGS of RV directly from clinical samples, which was the reason for the high price. WGS was considered necessary because RV evolves rapidly through homologous and heterologous genetic recombination,[62,70,785] a finding that was established in this study as well as others; hence, WGS is the best available mechanism to identify RV type accurately and correlate it with pathognomy.[62,70,785]

Finding the appropriate laboratory with which collaboration was possible took three years. The cost was cheaper with this laboratory than with the others' but still relatively expensive. However, they could do two things that the other laboratories cannot: use clinical samples without the need for prior laboratory processing by the researcher, and analyse data within a fortnight, rather than several months. In order to be able to pay for this collaborative service, the researcher offered to reduce the employment contract by half such that, the final year of the researcher's contract would then only last for six months. However, the WGS laboratory made a last minute stipulation, which was to have the names of their team listed as top authors in any publications that arise from their work, and they made this non-negotiable. This was not only unacceptable to the researcher but also unfair to the others who contributed to the TraVerse study. In the end, therefore, WGS was not done and the researcher was out of a job sooner than expected; all these would not have been an issue had there been grant funds available. Snell et al. (2017) suggested that the reasons why funding in respiratory research is hard to come by in the UK are, substandard collaborative efforts, unhelpfulness in advocating colleagues' grant proposals, and dissonance in promoting respiratory research agenda.[36] Clearly, this has to change.

It is important to note that, although a few difficulties were encountered in carrying out the TraVerse study, they were nevertheless considered mere nuisances that cannot be helped. Immense support was provided by the supervisory team, the review panel, the paediatrics team, and the research and administrative staff. All these made the TraVerse study an altogether positive research experience, of which one may be proud.

11.6 Conclusions and Recommendations

The TraVerse method of natural transmission was evaluated in this study, in relation to viruses relevant to the common cold including, RV, EV, Flu, HCoV, HAdV, RSV, hMPV and PIV. It was found to be fit-for-purpose in ascertaining respiratory disease spread that may have implications in future pandemics by large droplet, hand contact, self-inoculation, and fomites. Regrettably, the contributions of aerosol transmission were not appropriately evaluated, due to financial restrictions. Thus, it is recommended that TraVerse be further evaluated, to include aerosol measurement and to ascertain the reproducibility of the results established in this study.

TraVerse results include, large droplet transmission rates of 24% for RV, 5% for RSVB and 4% for EV, hand-to-hand transmission rates of 19% for RV and 18% for RSVB, self-inoculation rates of 100% for EV, 67% for RV, and 50% for RSVB, and fomite transmission rates of 33% for HAdV, 20% for EV, and 8% for RV. It was suggested here that hand and fomite transmission rates, particularly those of EV and HAdV, may be promoted by childhood mouthing behavior; hence, it is recommended that hand disinfection of paediatric patients be considered in devising intervention practices, rather than just the hands of hospital staff and visitors.

Transmission rates that resulted in host virus shedding were not elucidated for Flu, HAdV, HCoV, hMPV, PIV, and RSVA by any mechanism evaluated using the TraVerse method. Nevertheless, clinical illness was observed in healthy adults at rates of 100% for HAdV, HCoV1, and PIV1, 75% for PIV3, 67% for hMPV, 50% for

FluA(H3), and 29% for RSVA; thereby, suggesting that although shedding was not observed, presumptive transmission of disease nonetheless occurred. The reasons given in this study for the non-detection of viruses were, host shedding at very low levels that are not detectable by PCR, host shedding at levels detectable by PCR, but lower than the lowest quantitation standard value of 10^3 virus particles/ mL, and probable aerosol transmission.

This study further established that, contrary to general assumptions, healthy young adults are susceptible to severe disease manifestations that result in high symptom scores and long durations of illness. These were particularly noticeable in FluA (H3), HAdV, hMPV and PIV1 infections. It was also ascertained that, even though healthy young adults shed viruses and experienced severe illness, they were still not inclined to stay home, take an antipyretic, or seek a medical consultation. One possible reason put forward was that healthy adults have robust immune systems that respond appropriately to infection, so they are able to tolerate their symptoms better than children, whose immune systems respond either weakly or aggressively to a disease and leads to hospitalisations. However, studies that include serology tests would be better placed to comment on these. Therefore, it is strongly recommended here that hospital infection control policies involving the forced absence of symptomatic staff should be continued, particularly in high-risk wards, in case the staff are shedding transmissible viruses at very low levels. Asymptomatic infections were also observed in 5% of healthy adult hosts infected with RV. Since asymptomatic shedding promotes the silent nosocomial transmission of viruses, the rapid swab testing of staff is further recommended here as part of standard hospital infection control programmes.

The findings of note in this study were that: 1) RV-B causes mild infections, and is considerably more predominant in healthy young adults than paediatric patients, 2) RV-C is primarily a paediatric infection, although RV-A is the type most often transmitted, 3) in children, febrile convulsion is significantly associated with EV, and fever with HAdV and PIV3 infections, 4) RSVB transmission is primarily due to direct hand-to-hand-to-face contact, rather than through the more commonly suggested hand-to-fomite-to face contact, 5) hMPV is implicated in severe infections in healthy young adults, however, more data are needed to verify this

finding. It is, therefore, suggested that hMPV be included in routine diagnostic testing for respiratory infections, 6) RSV and PIV subtypes had varying infection rates, transmission mechanisms, and clinical manifestations, which provide further support for the need for a diagnostic assay that is able to subtype viruses, and 7) RSVA was more likely to be found on the hands of sources that are shedding the virus, had a shorter incubation period and longer durations of shedding, and was not transmitted to adult hosts. Taken altogether, this suggested the possibility of RSVA hand transmission from children to children and RSVB hand transmission from children to adults, which have implications for nosocomial RSV infections.

It was with great disappointment that the researcher was not able to ascertain Flu shedding rates that could be of benefit to current pandemic preparedness plans; however, it is suggested that the absence of transmission in this study may nonetheless provide support to the increasing supposition that Flu is primarily transmitted through aerosols.

In order to increase the chances of an accurate diagnosis of infection, flocked swabs were used in the sample collection and the most highly sophisticated RT-PCR method available, at the time of the study, was employed in diagnostics. It is recommended here that flocked swabs replace NPAs as standard in paediatric respiratory sample collection because NPAs inflict unnecessary pain on patients and add very little value to an accurate diagnosis. It is also recommended that, where finances are of no object, the use of quantitative, multiplex, real-time RT-PCR assays be the standard in diagnostic laboratories, for optimized results that can have a direct impact on patient management.

Finally, there were difficulties encountered by the researcher in this study that was brought to the reader's attention, so that they may be avoided in future research. They included difficulties in paediatric patient recruitment, logistics, the participation of healthy young adult minorities in research, and funding. When cogitating about these challenges, the researcher concluded that, with some amount of flexibility and commitment to the research, the challenges were tolerable and, in hindsight, amounted to nothing more than nuisances. However, with a great deal of support from supervisors, progress review panel members,

clinical staff and administrative and research services, the difficulties became relatively surmountable. For these, the researcher gives enormous thanks.

12 Tables

Table 3 Host Diary Card

Diary Card Parameters	No. Hosts reported (%)[j]
Absenteeism (Work/ University)	6 (7.1)
Antipyretic Use	12 (14)
GP Consultation	3 (3.6)
Oral Temp. ≥37.8˚C	4 (4.8)
Chest Pain	3 (3.6)
Chills	6 (7.1)
Cough, Dry	25 (30)
Cough, Productive	10 (12)
Decrease Appetite	11 (13)
Difficulty Breathing	3 (3.6)
Eye Pain	10 (12)
Facial Pain	6 (7.1)
Fatigue	26 (31)
Fever	8 (9.5)
Headache	31 (37)
Hoarseness	19 (23)
Malaise	17 (20)
Myalgia	15 (18)
Nasal Congestion	53 (63)
Runny Nose	47 (56)
Sneezing	30 (36)
Shortness of Breath	4 (4.8)
Sore Throat	39 (46)
Thick Nasal Discharge	18 (21)
Wheeze	2 (2.4)

[j] Symptomatic hosts (n=84)

Table 4 PCR Panel 1[k]

Primer/ Probe	Sequence	Working Conc. pmol/µL	Stock Vol. (µL)	Final Vol. (µL)	Vol. H_2O (µL)	Unit Vol. (µL)
AMF	5'-GAG TCT TCT AAC MGA GGT CGA AAC GTA-3'	20	50	250	200	0.5
AMR	5'-GGG CAC GGT GAG CGT RAA-3'	20	100	500	400	1.0
AH3F	5'-CCT TTT TGT TGA ACG CAG CAA-3'	50	250	500	250	1.0
AH3R	5'- CGG ATG AGG CAA CTA GTG ACC TA-3'	50	250	500	250	1.0
BNPF	5'-GCA GCT CTG ATG TCC ATC AAG CT-3'	20	16	80	64	0.16
BNPR	5'-CAG CTT GCT TGC TTA RAG CAA TAG GTC T-3'	20	16	80	64	0.16
MS2F	5'-TGG CAC TAC CCC TCT CCG TAT TCA CG-3'	20	10	50	40	0.1
MS2R	5'-GTA CGG GCG ACC CCA CGA TGA-C-3'	20	10	50	40	0.1
AM Probe	5'-JOE-TCC TGT CAC CTC TGA C-MGBNFQ-3'	10	20	200	180	0.4
AH3 Probe	5-FAM-CCT ACA GCA ACT GTT ACC-MGBNFQ-3	20	10	50	40	0.1
BNP Probe	5'-CY5-CCA GAT CTG GTC ATT GGR GCC CAR AAC TG-BHQ3-3'	3.3	3.3	100	96.7	0.2
MS2 Probe	5'-ROX-CAC ATC GAT AGA TCA AGG TGC CTA CAA-GC-BHQ2-3'	10	10	100	90	0.2
Total Vol. (µL)				2460	1714.7	4.92

[k] FluA(H1), FluA(H3), FluB and MS2 internal control Primer-Probe Mix (500 reactions)

Table 5 Panel 2 – RSVA and RSVB Primer-Probe Mix (500 reactions)

Primer / Probe	Sequence	Working Conc. pmol/µL	Stock Vol. (µL)	Final Vol. (µL)	Vol. H_2O (µL)	Unit Vol. (µL)
RSVF	5'-GGG WGG WGA AGC WGG ATT CTA CC-3'	20	50	250	200	0.5
RSVR	5'-ACC TCT RTA CTC TCC CAT TAT GCC TAG-3'	20	50	250	200	0.5
RSVA Probe	5'-FAM-TAT TAG GCA ATG CTG CTG-MBGNFQ-3'	10	10	100	90	0.2
RSVB Probe	5'-VIC-TCC TAG GCA ATG CAG CAG-MGBNFQ-3'	10	10	100	90	0.2
Total Vol. (µL)				700	580	1.4

Table 6 Panel 3 – RV and EV Primer-Probe Mix (500 reactions)

Primer / Probe	Sequence	Working Conc. pmol/µL	Stock Vol. (µL)	Final Vol. (µL)	Vol. H_2O (µL)	Unit Vol. (µL)
Ent/ RhinoF	5'-CGG CCC CTG AAT GYG GCT AA-3'	20	50	250	200	0.5
Ent/ RhinoR	5'-GAA ACA CGG ACA CCC AAA GTA-3'	20	50	250	200	0.5
Rhino Probe	5'-JOE-TCY GGG AYG GGA CCR ACT A-MGB-3'	10	15	150	135	0.3
Entero Probe	5'-6FAM-TCT GYR GCG GAA CCG ACT-MGB-3'	10	15	150	135	0.3
Total Vol. (µL)				800	670	1.6

Table 7 Panel 4 – HCoV Primer-Probe Mix (500 reactions)

Primer / Probe	Sequence	Working Conc. pmol/µL	Stock Vol. (µL)	Final Vol. (µL)	Vol. H_2O (µL)	Unit Vol. (µL)
CorF	5'- ATG GGT TGG GAY TAT CCI AAR TGT GA -3'	10	25	250	225	0.5
CorR1	5'- GCA GTA GTT GCA TCA CCA CTR CTA GT -3'	10	25	250	225	0.5
CorR2	5'- GCT GTA CTA GCR TCA CCA GAA GT -3'	10	25	250	225	0.5
CorR3	5'- GCT GTA GTT GCR (A TCA CCA GAA GT)-3'	10	25	250	225	0.5
CorR4	5'- AGC AGT TGT AGC ATC ACC GGA TGA T-3'	10	25	250	225	0.5
GP1 Probe	5'- FAM-TTR GGY TCT AAG CAT GTY A-MGB-3'	2	2	100	98	0.2
GP2 Probe	5'- JOE-CTT GCG AAT GAA TGY GC –MGB-3'	2	2	100	98	0.2
SARS Probe	5'- Cy5-CAG GTT AGC TAA CGA GTG TGC GCA AGT A-BHQ3-3'	2	2	100	98	0.2
Total Vol. (µL)				1800	1644	3.1

Table 8 PIVs 1 to 4 Primer-Probe Mix (500 reactions)

Primer / Probe	Sequence	Working Conc. pmol/µL	Stock Vol. (µL)	Final Vol. (µL)	Vol. H_2O (µL)	Unit Vol. (µL)
NP1F	5'-GCY CCT TTY ATA TGT ATA CTC AGA GAC CCA-3'	10	25	250	225	0.5
NP1R	5'-TGT TCT TCC AGT TAC ATA YTG TTG CAT AGC -3'	10	25	250	225	0.5
NP2F	5'-AAG TGY ATG ACT GCT CCT GAT CAR CC -3'	5	6.25	125	118.75	0.25
NP2R	5'-TTG CCA ATR TCT CCC ACC ATR GCA TA -3'	5	6.25	125	118.75	0.25
NP3F	5'-GCT CCT TTY ATC TGT ATC CTC AGA GAT CC -3'	10	25	250	225	0.5
NP3R	5'-TGA TCT TCC CGT CAC ATA CTG TTG CAT G -3'	10	25	250	225	0.5
NP4F	5'-AAA TGY ATG ACA GCT TAT GAT CAA CCC A -3'	5	6.25	125	118.75	0.25
NP4R	5'-TTT GCA ATR TCT CCC ACC ATR GCA TA -3'	5	6.25	125	118.75	0.25
NP1 Probe	5'-FAM-ATA RTT TCC AGG GGC AAA-MGB -3'	3	4.5	150	145.5	0.3
NP2 Probe	5'-Cy5-TCA GAA TGC CAT CCG CAA GTC AAT GG–BHQ3 -3'	1	0.5	50	49.5	0.1
NP3 Probe	5'- JOE-ATA GTT GCC TGG TGC GAA-MGB-3'	3	4.5	150	145.5	0.3
NP4 Probe	5'-ROX-CAG CTG ATA ARG TAG GTG CTT ATA CTA ACA G-BHQ2-3'	1	0.5	50	49.5	0.1
Total Vol. (µL)				1900	1765	3.8

Table 9 HAdV Primer-Probe Mix (500 reactions)

Primer/ Probe	Sequence	Working Conc. pmol/µL	Stock Vol. (µL)	Final Vol. (µL)	Vol. H_2O (µL)	Unit Vol. (µL)
AdenoF	5'-GCC CCA RTG GKC NTA CAT GCA CAT C-3'	20	50	250	200	0.5
AdenoR	5'-GCC ACX GTG GGR TTY CTR AAC TT–3'	20	50	250	200	0.5
Adeno Probe	5'-Cy5- TGC ACC AGA CCC GGR CTC AGR TAC TCC GA–BHQ3-3'	10	10	100	90	0.2
Total Vol. (µL)				600	490	1.2

Table 10 hMPV Primer-Probe Mix (500 reactions)

Primer / Probe	Sequence	Working Conc. pmol/µL	Stock Vol. (µL)	Final Vol. (µL)	Vol. H_2O (µL)	Unit Vol. (µL)
hMPVF	5'-CAT CAG GTA AYA TCC CAC AAA AYC AG–3'	10	25	250	225	0.5
hMPVR	5'-GTG AAT ATT AAR GCA CCT ACA CAT AAT AAR A–3'	10	25	250	225	0.5
hMPV Probe	5'-CY5–CCY TCA GCA CCA GAC ACA CC–BHQ3 -3'	3	4.5	150	145.5	0.3
Total Vol. (µL)				650	595.5	1.3

Table 11 FluA(pandemic H1) Primer-Probe Mix (500 reactions)

Primer / Probe	Sequence	Working Conc. pmol/µL	Stock Vol. (µL)	Final Vol. (µL)	Vol. H_2O (µL)	Unit Vol. (µL)
H1SwF	5'-TTA CCA GAT TTT GGC GAT CTA YT-3'	30	75	250	175	0.5
H1SwR	5'-CCA GGG AGA CTA SCA RTA CCA-3'	30	75	250	175	0.5

H1Sw Probe	5-6FAM-ACW GTC GCC AGT TC-MGBFQ-3’	5	31.25	625	593.75	1.25
Total Vol. (µL)				1125	943.75	2.25

Table 12 RNAseP Primer-Probe Mix (500 reactions)

Primer/ Probe	**Sequence**	**Working Conc. pmol/µL**	**Stock Vol. (µL)**	**Final Vol. (µL)**	**Vol. H_2O (µL)**	**Unit Vol. (µL)**
RNP-F	5’- AGA TTT GGA CCT GCG AGC G-3’	30	37.5	125	87.5	0.25
RNP-R	5’- GAG CGG CTG TCT CCA CAA GT-3’	30	37.5	125	87.5	0.25
RNP Probe	5’-FAM-TTC TGA CCT GAA GGC TCT GCG CG-3’	10	12.5	125	112.5	0.25
Total Vol. (µL)				375	287.5	0.75

Table 13 PCR Master Mix Recipes

	PCR-Grade Water (µL)	**2x Reaction Buffer (µL)**	**$MgSO_4$ (50mM) (µL)**	**Primer-Probe Mix (µL)**	**SS-III RT-PCR Enzyme (µL)**
Panel 1	1.78	12.5	0	4.92	0.8
Panel 2	3.8	12.5	1.5	1.4	0.8
Panel 3	4.1	12.5	1.0	1.6	0.8
Panel 4	2.1	12.5	1.5	3.1	0.8
Panel 5	1.4	12.5	1.5	3.8	0.8
HAdV	4	12.5	1.5	1.2	0.8
hMPV	4.4	12.5	1.0	1.3	0.8
Pandemic H1	4.45	12.5	0	2.25	0.8
RNAse P	5.95	12.5	0	0.75	0.8

Table 14 Source and Host Demographics

	Sources	**Hosts**
Mean Age (±SD)	26 mos. (18)	22 yrs. (3)
Median Age (IQR)	22 mos. (13-35)	22 yrs. (20-23)
Males (%)	67 (60)	50 (45)
Females (%)	44 (40)	61 (55)
Recent Flu Vaccine (%)	9 (8)	2 (2)
White/ Caucasian (%)	71 (64)	75 (68)
Asian (%)	23 (21)	23 (21)
Afro-Caribbean (%)	9 (8)	2 (2)
Oriental (%)	0	4 (4)
Other Ethnicity (%)	8 (7)	7 (6)

Table 15 Swab, Blood, and Environmental Samples Collected on Day 0 and Follow-up Days 1-10

	Nose-Throat	**Hand**	**Venous Blood**	**Face**	**Clothing**	**Toys**	**Bio Aerosol**	**Total**
Child Source	111	74						185
Adult Host	552	19	111	19				701
Adult Reference	11							11
Fomite					19	38[l]		57
Environ.							2	2
Total	674	93	111	19	19	38	2	956

[l] Pre-interaction (n=19) and Post-interaction (n=19)

Table 16 Virus Distributions in Source, Host, and Fomite.

	Child Source [Count (%)][m]		**Adult Host [Count (%)]**			**Fomites [Count (%)]**	
	Nose-throat[n]	Hands[o]	Nose-throat[p]	Hands[q]	Face[r]	Toys[s]	Clothing[t]
RV	67 (65)	16 (47)	14 (67)	3 (60)	2 (50)	1 (33)	1 (100)
EV	26 (25)	6 (18)	6 (29)		1 (25)	1 (33)	
RSVB	21 (20)	11 (32)	3 (13)	2 (40)	1 (25)		
HAdV	11 (11)	7 (21)				1 (33)	
PIV3	9 (9)	1 (3)					
RSVA	7 (7)	3 (9)	1 (4.2)				
hMPV	7 (7)	1 (3)					
FluA(H3)	4 (4)				1 (25)		
HCoV1[u]	4 (4)						
PIV1	2 (2)	1 (3)					
HCoV2[v]	1 (1)						
PIV4						1 (33)	

[m] % = Count / number of positives (n) x 100 (swabs may be positive for >1 virus)
[n] n=103 positive source nose-throat swabs out of 111 swabbed
[o] n=34 positive source hand swabs out of 74 swabbed
[p] n=21 positive host nose-throat swabs out of 111 swabbed
[q] n=5 positive host hand swabs out of 19 swabbed
[r] n=4 positivie host face swabs out of 19 swabbed
[s] n=3 positive post-interaction toys out of 19 swabbed
[t] n=1 positive host clothing out of 19 swabbed
[u] Strains NL63 and 229E
[v] Strains OC43 and HKU1

Table 17 Mono and Multiple Detections of RV Types Identified From Source, Host, and Toy Swabs[w]

	Paediatric Source RV (n=61)			**Adult Host RV (n=68)**		
Nose-Throat Swabs RV Type	**Mono**	**Multiple**	**Total (%)**	**Mono**	**Multiple**	**Total (%)**
RV-A	12	7	19 (31)	16		16 (24)
RV-B		4	4 (6.6)	2	41	43 (63)
RV-C	5	33	38 (62)	5	4	9 (13)
Hand Swabs RV Type	**Paediatric Source RV (n=16)**			**Adult Host RV (n=3)**		
RV-A	5	2	7 (44)	2	1	3 (100)
RV-C		9	9 (56)			
Toys and Face RV Type	**Toys Post-Interaction (n=1)**			**Adult Host Face RV (n=2)**		
RV-A				1		1 (50)
RV-C		1	1 (100)		1	1 (50)

Table 18 Median Viral Loads (copies/ mL) for Source, Host, and Fomite

	Source (IQR)		**Host (IQR)**			**Fomites**	
	Nose-throat	Hands	Nose-throat	Hands	Face	Toys	Clothing
EV	$5.0x10^3$ (10^3-10^4)	$5.0x10^3$ (10^3-10^3)	$5.0x10^3$ (10^3-10^3)		<$5.0x10^3$	<$5.0x10^3$	
FluA(H3)	$6.8x10^5$ (10^5-10^6)				$6.8x10^2$		
RSVA	$4.4x10^6$ (10^5-10^6)	$2.0x10^4$ (10^4-10^4)	$2.0x10^4$ (10^4-10^5)				
RSVB	$5.0x10^6$ (10^5-10^6)	$3.6x10^4$ (10^4-10^4)	$5.7x10^3$ (10^3-10^4)	$5.0x10^3$ (10^3-10^4)	$1.3x10^5$		
RV	$9.2x10^4$ (10^3-10^5)	$5.0x10^3$ (10^3-10^3)	$2.3x10^4$ (10^3-10^5)	$5.0x10^3$ (10^3-10^3)	<$5.0x10^3$ (10^3-10^3)	<$5.0x10^3$	<$5.0x10^3$

[w] n = number of RV samples sequenced

Table 19 TraVerse Natural Respiratory Virus Transmission Rates

	Large Drplt[x]	**Hand[y]**	**Self-Inoculation[z]**	**Fomite[aa]**	**Asymptomatic[bb]**	**Clinical Manifestations[cc]**
EV	3.9% (1/26)		100% (1/1)	20% (1/5)		
FluA (H3)						50% (1/2)
HAdV				33% (1/3)		100% (2/2)
HCoV1						100% (2/2)
hMPV						67% (2/3)
PIV1						100% (1/1)
PIV3						75% (3/4)
RSVA						29% (2/7)
RSVB	4.8% (1/21)	18% (2/11)	50% (1/2)			4.6% (1/22)
RV (All)	24% (16/67)	19% (3/16)	67% (2/3)	7.7% (1/13)	4.5% (3/67)	29% (8/28)
RV-A	12% (8/67)	13% (2/16)	50% (1/2)		33% (1/3)	38% (3/8)
RV-C	3.0% (2/67)					
RV-A+C	9.0% (6/67)	6.3% (1/16)	50% (1/2)	7.7% (1/13)	67% (2/3)	50% (4/8)

[x] Large droplet % transmission = no. of host that shed virus / no. of source that shed virus
[y] Hand-to-hand % transmission = no. of host with virus on hands / no. of source with virus on hand
[z] % Self-inoculation = no. of host with virus on hands / no. of host with virus on face
[aa] Fomite % transmission = no. of toys with virus / no. of source hands with virus
[bb] Asymptomatic % transmission = no. of asymptomatic host that shed virus / no. of source that shed virus
[cc] % Clinical manifestations = no. of virus-negative host with ILI / no. of source with mono-infection of virus

Table 20 Virus Incubation Periods and Durations of Shedding and Symptom Development

Virus	Shedding (Days)		Symptom Development (Days)	
	Incubation Median (IQR)	**Duration Median (IQR)**	**Incubation Median (IQR)**	**Duration Median (IQR)**
EV	3 (1 - 6)		1 (0 - 1)	8 (4 - 8)
RSVB	3 (3 - 5)	2 (3 - 5)	2 (1 - 4)	5 (4 - 6)
RV	5 (3 - 6)	4 (2 - 7)	1 (1 - 3)	4 (2 - 8)

Table 21 Paediatric Symptoms at ED/ CAU Presentation

Presenting Symptom	**No. Cases (%)**
Cough	109 (98)
Difficulty-in-breathing	105 (95)
Tachypnoea	97 (87)
Nasal discharge	94 (85)
Wheeze	94 (85)
Sneezing	80 (72)
Fevers/ sweats	65 (59)
Hoarseness	64 (58)
Chest recession	60 (54)
Tympanic temperature ≥37.8	27 (24)
Vomiting	27 (24)
Lung crackles	24 (22)
Diarrhoea	17 (15)
Febrile convulsions	8 (7)
Headaches	7 (6)

Table 22 Signs and Symptoms of Statistical Significance to Source Virus Infections (*p* values)[dd]

Signs and Symptoms	RV	EV	HCoV	RSVA	RSVA & RSVB	PIV3	PIV1 & PIV3	HAdV
Diarrhoea	0.018				0.044			
Febrile convulsion		0.019						
Fever							0.035	0.028
Temp ≥37.8°C	0.003				0.045	0.043	0.015	
Wheeze			0.029					
Overall Signs	<0.001			0.024				

Table 23 Signs and Symptoms of Statistical Significance to Hosts with ILI[ee]

Respiratory Symptoms & Systemic Signs	≥6 mean symptom score % (*p* value)[ff]	≥3 days % (*p* value)[gg]
Chills	23 (0.004)	11 (>0.05)
Cough, All	50 (<0.001)	27 (0.002)
Cough, Non-productive	64 (<0.001)	39 (0.009)
Cough, Productive	36 (<0.001)	16 (>0.05)
Decreased appetite	41 (<0.001)	18 (>0.05)
Difficulty Breathing	14 (0.016)	5.3 (>0.05)
Facial pain	23 (0.004)	11 (>0.05)
Fatigue	64 (<0.001)	39 (0.028)
Fever	27 (0.004)	12 (>0.05)
Headache	55 (>0.05)	46 (0.017)
Hoarseness	59 (<0.001)	32 (0.003)
Malaise	68 (<0.001)	30 (<0.001)
Myalgia	50 (<0.001)	25 (0.017)
Nasal Congestion	91 (0.001)	81 (<0.001)
Rhinorrhoea	77 (0.02)	68 (0.001)

[dd] Signs and Symptoms are those that discretely associate with the respective viruses
[ee] ILI = mean symptom severity score ≥6 and symptom duration ≥3 days
[ff] % = no. hosts with ILI with specific symptom / no. all hosts with specific symptom and mean severity score ≥6
[gg] % = no. hosts with ILI with specific symptom / no. all hosts with specific symptom and symptom duration ≥3 days

Shortness of Breath	18 (0.004)	7.0 (>0.05)
Sneeze	59 (0.011)	46 (0.006)
Sore throat	95 (<0.001)	60 (<0.001)
Thick Nasal Discharge	36 (>0.05)	30 (0.005)
Overall Signs	38 (<0.001)	21 (<0.001)
Overall Symptoms	51 (<0.001)	35 (<0.001)

Table 24 Signs and Symptoms of Statistical Significance to Host Virus Infections[hh]

Signs and Symptoms	**RV % (*p* value)[ii]**	**RSVB % (*p* value)[jj]**	**RSVA & RSVB % (*p* value)[kk]**
Cough, dry			33 (0.025)
Cough, productive	60 (0.045)		
Fatigue	46 (0.046)		
Hoarseness		16 (0.037)	21 (0.008)
Malaise			18 (0.049)
Myalgia		20 (0.018)	20 (0.034)
Sore throat			13 (0.021)
Overall Respiratory	38 (0.001)	8.4 (0.002)	11 (<0.001)
Overall Systemic	4.1 (0.023)	10 (0.005)	12 (0.001)

[hh] Symptomatic RV-positive host (n=33); Symptomatic RSVB-positive host (n=5); Symptomatic RSVA & RSVB-positive host (n=6)
[ii] % = no. RV-positive hosts with specific symptom /no. all hosts with specific symptom
[jj] % = no. RSVB-positive hosts with specific symptom / no. all hosts with specific symptom
[kk] % = no. RSVA & RSVB-positive hosts with specific symptom / no. all hosts with specific symptom

Table 25 Signs and Symptoms Reported by Seven Symptomatic Reference Adults

Presenting Symptom	No. Cases (%)[ll]
Rhinorrhoea	7 (100)
Nasal Congestion	5 (71)
Sneeze	4 (57)
Malaise	3 (43)
Fatigue	3 (43)
Sore eyes	2 (29)
Headache	2 (29)
Non-productive cough	2 (29)
Sore throat	2 (29)
Thick nasal discharge	2 (29)
Hoarseness	1 (14)
Myalgia	1 (14)
Productive cough	1 (14)

Table 26 Statistically Significant Risks of Sources Transferring a Specific Virus from Their Nose and Throat to Their Hands

	EV	PIV1	RSVA	RSVB	RV
Risk % (95% CI)	23 (11 – 42)	50 (9.5 – 91)	43 (16 – 75)	45 (27 -65)	21 (13 – 32)
Risk Ratio (95% CI)	39.5 (2.29 – 683)	72.5 (3.30 – 1590)	57.9 (3.21 – 1040)	23.6 (3.22 – 174)	4.6 (1.1 – 19)
p Value	<0.001	0.040	0.001	<0.001	0.015

[ll] % = no. references with specific symptom / seven (total symptomatic references)

Table 27 Risks to Hosts of Contact with Sources That Have RSVB on Their Hands

RSVB	**Host Hand Contamination**	**Host Face Contamination**	**Host Symptoms**	**Host Systemic Signs**
Risk % (95% CI)	18 (4.0 – 49)	50 (9.5 – 91)	8.3 (4.4 – 15)	7.3 (3.5 – 14)
Risk Ratio (95% CI)	23.1 (1.11 – 479)	72.5 (3.30- 1590)	5.2 (2.3 – 12)	9.2 (3.1 – 28)
p Value	0.037	0.040	<0.001	<0.001

Table 28 Temperatures and Relative Humidity Readings Taken Daily and During Interactions

	No. Readings	Median (IQR)
Temperature (°C)		
Daily Reading	401	22 (21 – 23)
During Interaction	58	22 (22 – 23)
Relative Humidity (%)		
Daily Reading	401	22 (21 – 23)
During Interaction	58	23 (20 – 24)

13 Figures

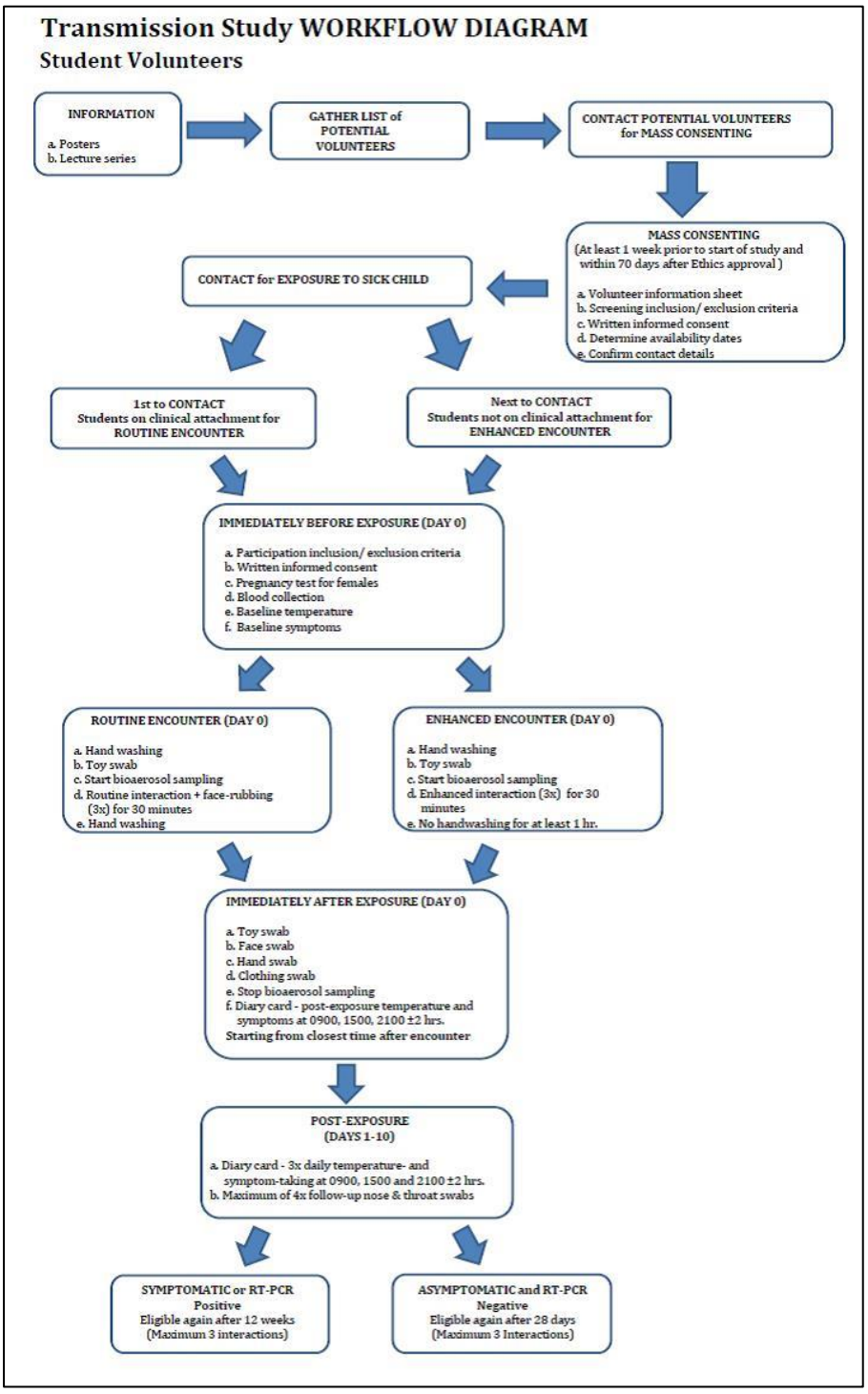

Figure 8 Workflow for healthy young adult volunteers

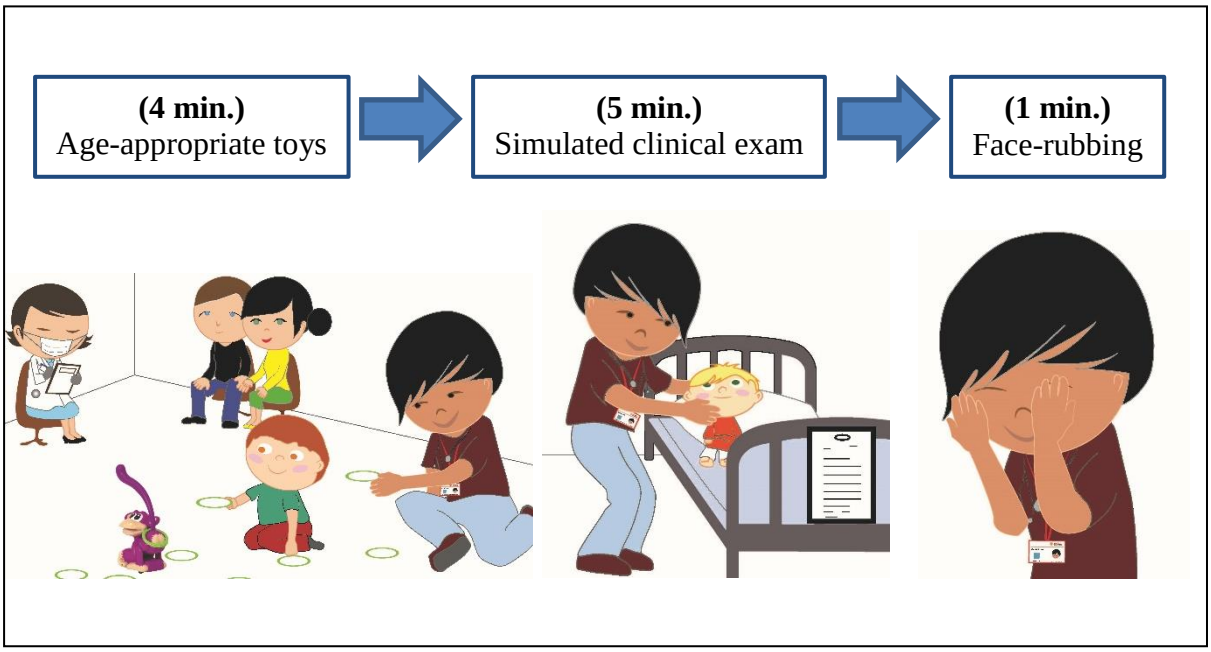

Figure 9 TraVerse method of natural respiratory virus transmission (3 x 10-min. cycles)

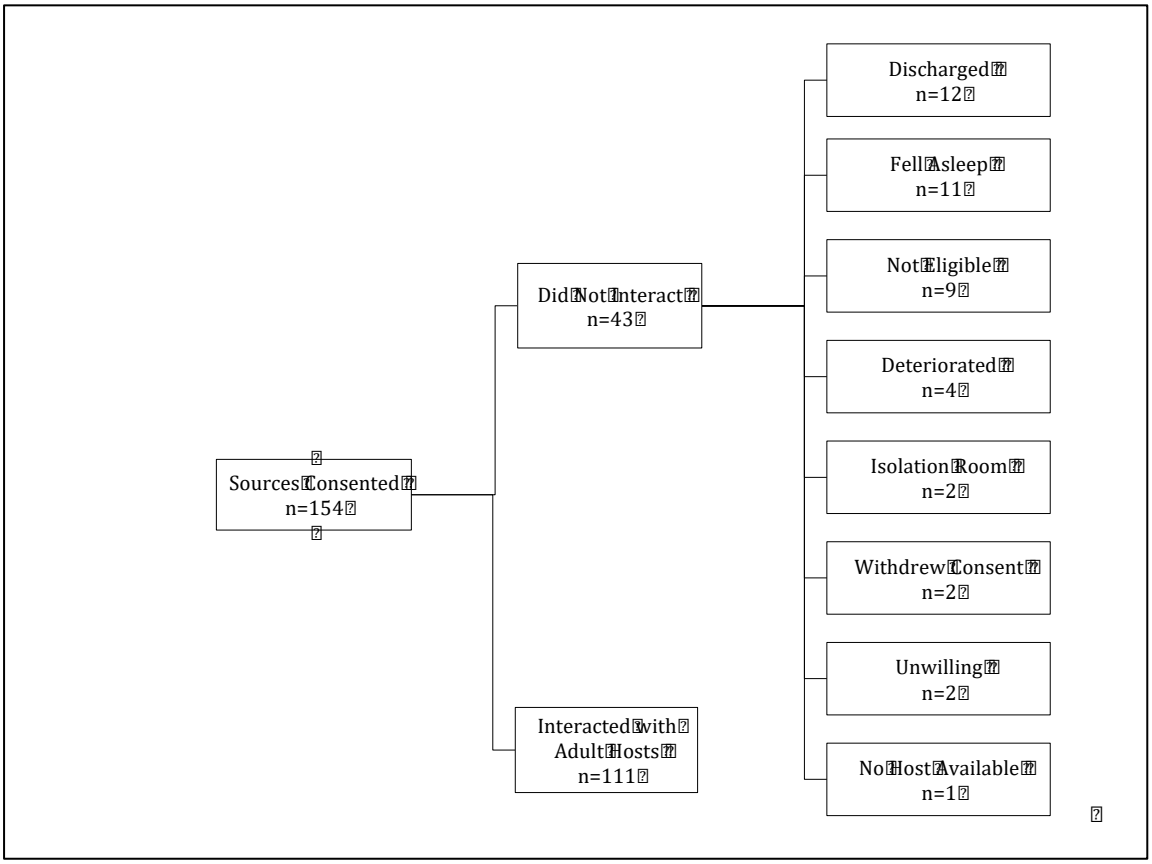

Figure 10 Flowchart of Paediatric Recruitment

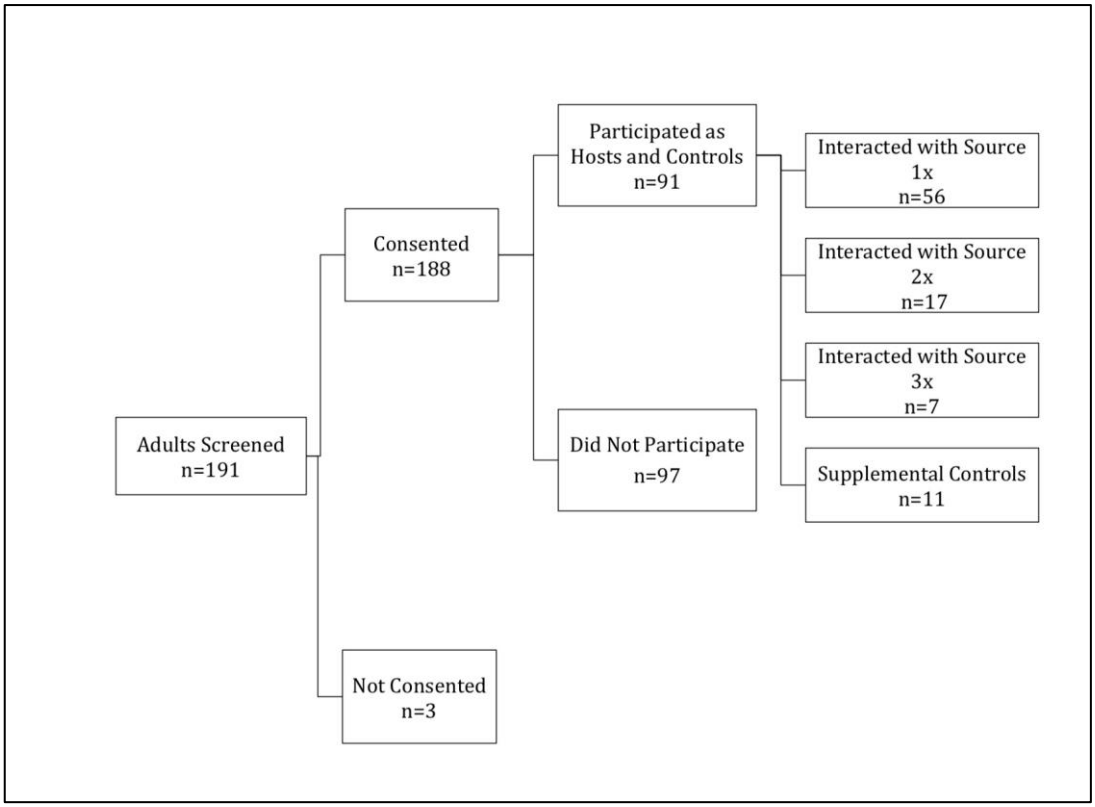

Figure 11 Flowchart of Healthy Young Adult Recruitment

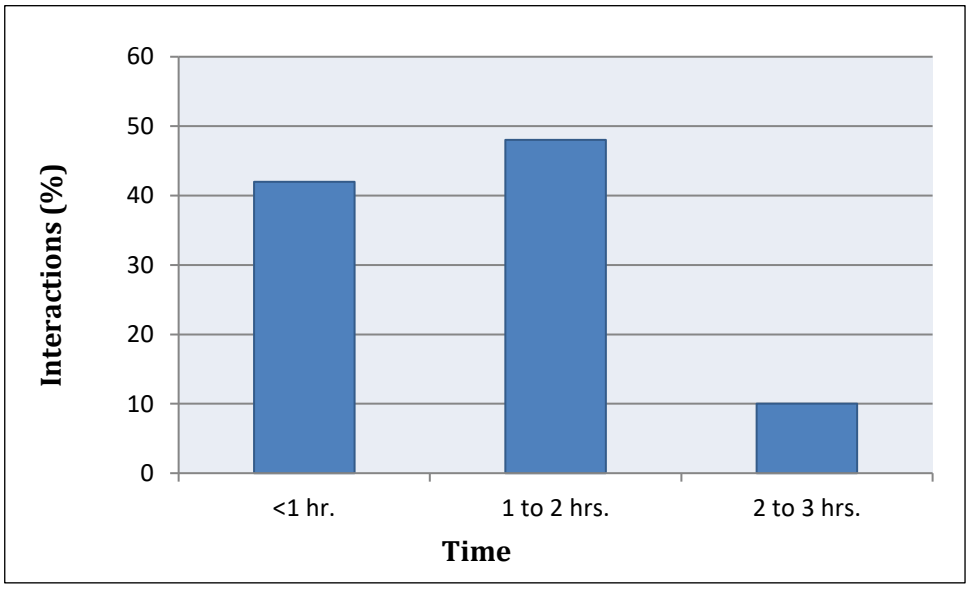

Figure 12 Time from Source Consent to Source-Host Interaction

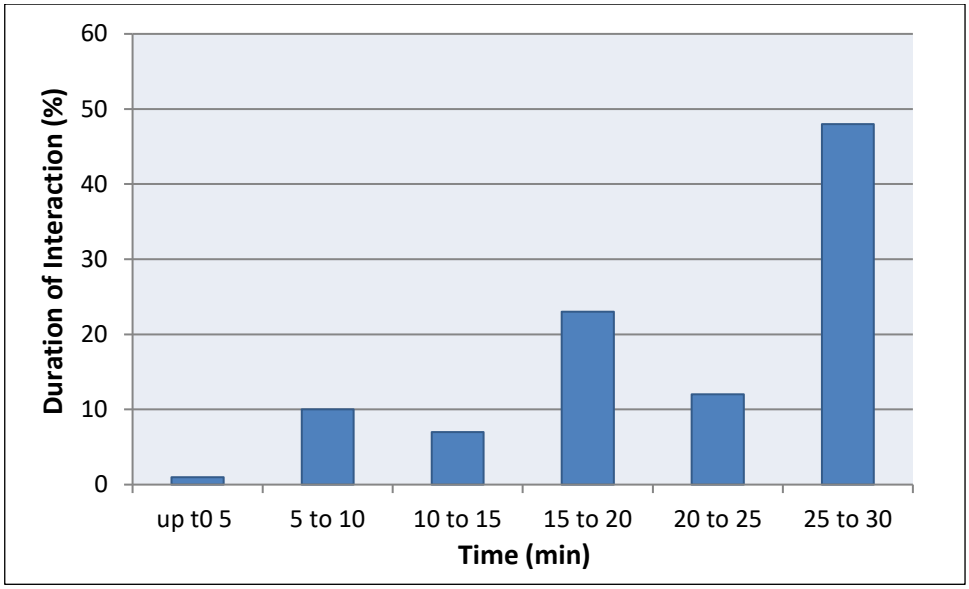

Figure 13 Duration of Source-Host Interactions

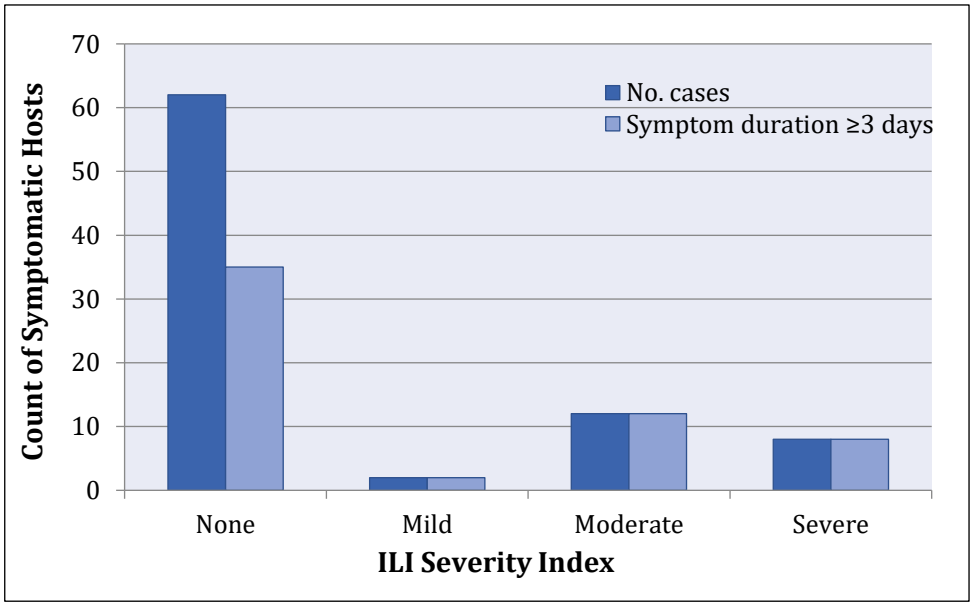

Figure 14 Incidence, Severity, and Duration of Influenza-like-illness in Healthy Adult Hosts[mm]

[mm] Mean symptom score indices (None: score <6. Mild: score=6. Moderate: score=7 to 11. Severe: score≥12)

14 Appendix

A1. Paediatric Inclusion Criteria

Inclusion criteria

a. Age 0 to ≤6 years
b. Parent or legal guardian are fluent and literate in English
c. Parent or legal guardian willing to provide informed written consent
d. Present to GP or to the LRI ED/ CAU with at least 2 of any respiratory symptoms or signs (see below), with onset within 216 hours (9 days) of recruitment into the study

Defined clinical signs or symptoms

Respiratory

Nasal discharge (clear or mucopurulent)

Sneezing

Hoarseness

Cough

Difficulty in breathing (DIB)

Rapid breathing

Chest recession (from case notes)

Crackles on examination (from case notes)

Wheeze (from case notes)

Systemic

Headache

Febrile convulsions

Fevers/sweats

Tympanic temperature ≥37.8^{o}C (from case notes)

Diarrhoea

Vomiting

A2. Paediatric Exclusion Criteria

Exclusion Criteria

a. Written informed consent unavailable from a parent or legal guardian
b. Unable to obtain nose & throat swabs for analysis
c. Unable to obtain hand swabs for analysis
d. Pre-existing conditions (unrepaired structural cardiac abnormalities, known inborn errors of metabolism, born before 32 weeks gestation)
e. Severe illness as determined by the Clinical staff in the ED/CAU/Paediatric wards
f. Duration of illness ≥234 hours at the time of exposure to a healthy adult volunteer so that the exposure can be completed within 240 (10 days) hours of onset of illness

A3. Healthy Young Adult Inclusion Criteria

Inclusion Criteria - Hosts

a. Fluent and literate in English
b. Willing to give informed written consent
c. 18 to ≤35 years of age
d. Will avoid direct contact with immune-suppressed patients for the duration of any symptomatic respiratory illness following exposure to a potentially infected child
e. Can be contacted by mobile phone, e-mail or text message

Inclusion Criteria – References

a. Fluent and literate in English
b. Willing to give informed written consent
c. 18 to <35 years of age
d. Can be contacted by mobile phone, e-mail or text message.

A4. Healthy Young Adult Exclusion Criteria

Exclusion Criteria (At Screening)

a. Unable to provide written informed consent
b. Immune function disorder
    i. Hypogammaglobulinaemia
    ii. Receipt of oral immunosuppressive drugs or other drugs listed in section 8 of the British National Formulary (BNF) or chloroquine, gold or penicillamine or other drugs listed in section 10.1.3 of the BNF to suppress a chronic disease process, or have received in the last 6 months radiotherapy or chemotherapy (Note: long-term, inhaled steroids for asthma management is acceptable)
    iii. Receipt of immunostimulants or interferon
    iv. Receipt of an immunoglobulin preparation, blood products, and/or plasma derivatives within 3 months of the study

v. Anyone at high risk of developing immunocompromising condition

vi. Received radiotherapy or chemotherapy during the 6 months preceding the study

c. Underlying chronic medical condition known to increase risk of respiratory virus related complications [lung disease (including asthma), chronic cardiovascular disease, diabetes mellitus, chronic neurological disorder)

d. Inability or contraindication to collection of nose & throat swabs

e. Pregnancy or breast-feeding

Additional Exclusion Criteria (Immediately Before Interaction)

a. Symptomatic respiratory tract illness during the preceding 14 days

b. Symptomatic respiratory tract illness on day 0, before exposure to a symptomatic child

c. Oral temperature ≥37.4°C

d. Unable to provide nose & throat swabs for respiratory virus PCR on at least four (preferably five) occasions – on day 0 (immediately preceding the exposure); and post-exposure on day 1; at least once between days 3, 4 and 5; once on either days 6 or 7; and at least once between days 8, 9 and 10

e. Unable to provide a blood sample

f. The exposure with a potentially infected child cannot be completed within 240 hours of onset of illness in the child

g. Cannot be contacted by mobile phone, e-mail or text message

Exclusion Criteria – References

a. Unable to provide informed written consent

b. Inability or contraindication to collection of nose & throat swabs

c. Pregnancy or breast-feeding

d. Have been involved in an interaction with potential infected child in the study in ≤28 days

15 Bibliography

1. Turner RB. 58. the common cold. In: Bennett JE, Dolin R, Blaser MJ, eds. *Mandell, douglas, and bennett's principles and practice of infectious diseases.* 8th ed. Philadelphia: Elsevier Saunders; 2015:748-752.

2. Wat D. The common cold: A review of the literature. *Eur J Intern Med*. 2004;15(2):79-88.

3. Vital Strategies. Four million deaths each year caused by acute respiratory infections - new atlas details pandemic. https://www.vitalstrategies.org/newsroom/four-million-deaths-each-year-caused-by-acute-respiratory-infections-new-at. Updated 9 Nov., 2010. Accessed Aprill/ 27, 2017.

4. Carter E. Fighting the cold war: David Tyrrell and the common cold. . 2013.

5. Tyrrell DAJ, Fielder M. *Cold wars: The fight against the commmon cold.* Oxford: Oxford University Press; 2002.

6. Roden AT. Variations in the clinical pattern of experimentally induced colds. *J Hyg (Lond)*. 1963;61:231-246.

7. Tyrrell DAJ. Studies of rhinoviruses and coronaviruses at the common cold unit, salisbury, wiltshire. *Postgrad Med J*. 1979;55(640):117-121.

8. Tyrrell DAJ, Cohen S, Schlarb JE. Signs and symptoms in common colds. *Epidemiol Infect*. 1993;111(1):143-156.

9. Jacobs SE, Lamson DM, St George K, Walsh TJ. Human rhinoviruses. *Clin Microbiol Rev*. 2013;26(1):135-162.

10. D'Alessio DJ, Meschievitz CK, Peterson JA, Dick CR, Dick EC. Short-duration exposure and the transmission of rhinoviral colds. *J Infect Dis*. 1984;150(2):189-194.

11. Gwaltney JM,Jr. Epidemiology of the common cold. *Ann N Y Acad Sci*. 1980;353:54-60.

12. Killingley B, Enstone JE, Greatorex J, et al. Use of a human influenza challenge model to assess person-to-person transmission: Proof-of-concept study. *J Infect Dis*. 2012;205(1):35-43.

13. Meschievitz CK, Schultz SB, Dick EC. A model for obtaining predictable natural transmission of rhinoviruses in human volunteers. *J Infect Dis*. 1984;150(2):195-201.

14. Smorodintseff AA, Tushinsky MD, Drobyshevskaya AI, Korovin AA, Osetroff AI. Investigation of volunteers infected with the influenza virus. *The American Journal of Medical Sciences*. 1937;194(2):159-170.

15. Hurwitz ES, Gunn WJ, Pinsky PF, Schonberger LB. Risk of respiratory illness associated with day-care attendance: A nationwide study. *Pediatrics*. 1991;87(1):62-69.

16. Monto AS, Ullman BM. Acute respiratory illness in an american community. the Tecumseh study. *JAMA*. 1974;227(2):164-169.

17. Mackay IM, Lambert SB, Faux CE, et al. Community-wide, contemporaneous circulation of a broad spectrum of human rhinoviruses in healthy australian preschool-aged children during a 12-month period. *J Infect Dis*. 2013;207(9):1433-1441.

18. Martin ET, Fairchok MP, Kuypers J, et al. Frequent and prolonged shedding of bocavirus in young children attending daycare. *J Infect Dis*. 2010;201(11):1625-1632.

19. Williams JV, Wang CK, Yang CF, et al. The role of human metapneumovirus in upper respiratory tract infections in children: A 20-year experience. *J Infect Dis*. 2006;193(3):387-395.

20. Chonmaitree T, Revai K, Grady JJ, et al. Viral upper respiratory tract infection and otitis media complication in young children. *Clin Infect Dis*. 2008;46(6):815-823.

21. Martin ET, Fairchok MP, Stednick ZJ, Kuypers J, Englund JA. Epidemiology of multiple respiratory viruses in childcare attendees. *J Infect Dis*. 2013;207(6):982-989.

22. Tyrrell DA, Cohen S, Schlarb JE. Signs and symptoms in common colds. *Epidemiol Infect*. 1993;111(1):143-156.

23. Dick EC, Jennings LC, Mink KA, Wartgow CD, Inhorn SL. Aerosol transmission of rhinovirus colds. *J Infect Dis*. 1987;156(3):442-448.

24. Gwaltney JM,Jr, Moskalski PB, Hendley JO. Hand-to-hand transmission of rhinovirus colds. *Ann Intern Med*. 1978;88(4):463-467.

25. Gwaltney JM,Jr, Hendley JO. Transmission of experimental rhinovirus infection by contaminated surfaces. *Am J Epidemiol*. 1982;116(5):828-833.

26. Hall CB, Douglas RG,Jr. Modes of transmission of respiratory syncytial virus. *J Pediatr*. 1981;99(1):100-103.

27. Tellier R. Review of aerosol transmission of influenza A virus. *Emerg Infect Dis*. 2006;12(11):1657-1662.

28. Fendrick AM, Monto AS, Nightengale B, Sarnes M. The economic burden of non-influenza-related viral respiratory tract infection in the united states. *Arch Intern Med*. 2003;163(4):487-494.

29. Heikkinen T, Jarvinen A. The common cold. *Lancet*. 2003;361(9351):51-59.

30. Armstrong D, Grimwood K, Carlin JB, et al. Severe viral respiratory infections in infants with cystic fibrosis. *Pediatr Pulmonol*. 1998;26(6):371-379.

31. Greenberg SB. Respiratory viral infections in adults. *Curr Opin Pulm Med*. 2002;8(3):201-208.

32. Hashem M, Hall CB. Respiratory syncytial virus in healthy adults: The cost of a cold. *J Clin Virol*. 2003;27(1):14-21.

33. Ralston SL, Lieberthal AS, Meissner HC, et al. Clinical practice guideline: The diagnosis, management, and prevention of bronchiolitis. *Pediatrics*. 2014;134(5):e1474-502.

34. Lowen AC, Mubareka S, Steel J, Palese P. Influenza virus transmission is dependent on relative humidity and temperature. *PLoS Pathog*. 2007;3(10):1470-1476.

35. Gwaltney JM,Jr, Hendley JO. Transmission of experimental rhinovirus infection by contaminated surfaces. *Am J Epidemiol*. 1982;116(5):828-833.

36. Snell N, Jarrold I, Holgate S. The current state of respiratory research in the UK. *Thorax*. 2015;70(11):1011-1013.

37. World Health Organization. Acute respiratory infections: The forgotten pandemic. *Bull World Health Organ*. 1998;76(1):101-3, 105-7.

38. World Health Organization. *International health regulations (2005).* Third ed. Geneva: WHO Press; 2005.

39. World Health Organization. *Pandemic influenza preparedness and response: WHO guidance document.* Geneva: WHO Press; 2009.

40. Roos R. GAO details spending on, lessons from 2009 pandemic. http://www.cidrap.umn.edu/news-perspective/2011/06/gao-details-spending-lessons-2009-pandemic. Updated 28 June, 2011. Accessed 27 April, 2017.

41. Al-Tawfiq JA, Assiri A, Memish ZA. Middle east respiratory syndrome novel corona MERS-CoV infection. epidemiology and outcome update. *Saudi Med J*. 2013;34(10):991-994.

42. Breban R, Riou J, Fontanet A. Interhuman transmissibility of middle east respiratory syndrome coronavirus: Estimation of pandemic risk. *Lancet*. 2013;382(9893):694-699.

43. Carballal G, Videla C, Misirlian A, Requeijo PV, Aguilar Mdel C. Adenovirus type 7 associated with severe and fatal acute lower respiratory infections in argentine children. *BMC Pediatr*. 2002;2:6.

44. Esposito DH, Gardner TJ, Schneider E, et al. Outbreak of pneumonia associated with emergent human adenovirus serotype 14--southeast alaska, 2008. *J Infect Dis*. 2010;202(2):214-222.

45. Brandt CD, Kim HW, Vargosko AJ, et al. Infections in 18,000 infants and children in a controlled study of respiratory tract disease. I. adenovirus pathogenicity in relation to serologic type and illness syndrome. *Am J Epidemiol*. 1969;90(6):484-500.

46. Frank AL, Taber LH, Wells CR, Wells JM, Glezen WP, Paredes A. Patterns of shedding of myxoviruses and paramyxoviruses in children. *J Infect Dis*. 1981;144(5):433-441.

47. Hall CB, Douglas RG,Jr, Geiman JM. Respiratory syncytial virus infections in infants: Quantitation and duration of shedding. *J Pediatr*. 1976;89(1):11-15.

48. Pallansch MA, Oberste MS, Whitton JL. Chapter 17 enteroviruses: Polioviruses, coxsackie viruses, echoviruses, and newer enteroviruses. In: Knipe DM, Howley PM, eds. *Fields virology.* Sixth ed. Philadelphia: Wolters Kluwer/ Lippincott Williams & Wilkins; 2013:490-530.

49. Hall WJ, Hall CB, Speers DM. Respiratory syncytial virus infection in adults: Clinical, virologic, and serial pulmonary function studies. *Ann Intern Med*. 1978;88(2):203-205.

50. Racaniello VR. Chapter 16 *picornaviridae:* the viruses and their replication. In: Knipe DM, Howley PM, eds. *Fields virology.* Sixth ed. Philadelphia: Wolters Kluwer/ Lippincott Williams & Wilkins; 2013:453-489.

51. Peltola V, Waris M, Osterback R, Susi P, Hyypia T, Ruuskanen O. Clinical effects of rhinovirus infections. *J Clin Virol*. 2008;43(4):411-414.

52. Eccles R. Understanding the symptoms of the common cold and influenza. *Lancet Infect Dis*. 2005;5(11):718-725.

53. Falsey AR, Treanor JJ, Betts RF, Walsh EE. Viral respiratory infections in the institutionalised elderly: Clinical and epidemiologic findings. *Journal of the American Geriatrics Society*. 1992;40:115.

54. Andreoletti L, Lesay M, Deschildre A, Lambert V, Dewilde A, Wattre P. Differential detection of rhinoviruses and enteroviruses RNA sequences associated with classical immunofluorescence assay detection of respiratory virus antigens in nasopharyngeal swabs from infants with bronchiolitis. *J Med Virol*. 2000;61(3):341-346.

55. Collinson J, Nicholson KG, Cancio E, et al. Effects of upper respiratory tract infections in patients with cystic fibrosis. *Thorax*. 1996;51(11):1115-1122.

56. Chung JY, Han TH, Kim SW, Hwang ES. Respiratory picornavirus infections in korean children with lower respiratory tract infections. *Scand J Infect Dis*. 2007;39(3):250-254.

57. Johnston SL, Pattemore PK, Sanderson G, et al. Community study of role of viral infections in exacerbations of asthma in 9-11 year old children. *BMJ*. 1995;310(6989):1225-1229.

58. Juven T, Mertsola J, Waris M, et al. Etiology of community-acquired pneumonia in 254 hospitalized children. *Pediatr Infect Dis J*. 2000;19(4):293-298.

59. Smyth AR, Smyth RL, Tong CY, Hart CA, Heaf DP. Effect of respiratory virus infections including rhinovirus on clinical status in cystic fibrosis. *Arch Dis Child*. 1995;73(2):117-120.

60. Papadopoulos NG, Xatzipsalti M, Jonhston SL. Rhinoviruses. In: A.J. Zuckerman, J.E. Banatvala, B.D. Schoub, P.D. Griffiths, and P. Mortimer, ed. *Principles and practice of clinical virology.* 6th ed. Chichester: John Wiley & Sons, Ltd.; 2009:489.

61. Waman VP, Kolekar PS, Kale MM, Kulkarni-Kale U. Population structure and evolution of rhinoviruses. *PLoS One*. 2014;9(2):e88981.

62. Palmenberg AC, Spiro D, Kuzmickas R, et al. Sequencing and analyses of all known human rhinovirus genomes reveal structure and evolution. *Science*. 2009;324(5923):55-59.

63. Gern JE, Palmenberg AC. Chapter 18 rhinoviruses. In: Knipe DM, Howley PM, eds. *Fields virology.* Sixth ed. Philadelphia: Wolters Kluwer/ Lippincott Williams & Wilkins; 2013:531-549.

64. Huang T, Wang W, Bessaud M, et al. Evidence of recombination and genetic diversity in human rhinoviruses in children with acute respiratory infection. *PLoS One*. 2009;4(7):e6355.

65. McIntyre CL, McWilliam Leitch EC, Savolainen-Kopra C, Hovi T, Simmonds P. Analysis of genetic diversity and sites of recombination in human rhinovirus species C. *J Virol*. 2010;84(19):10297-10310.

66. Palmenberg AC, Rathe JA, Liggett SB. Analysis of the complete genome sequences of human rhinovirus. *J Allergy Clin Immunol*. 2010;125(6):1190-9; quiz 1200-1.

67. Ison MG, Hayden FG, Kaiser L, Corey L, Boeckh M. Rhinovirus infections in hematopoietic stem cell transplant recipients with pneumonia. *Clin Infect Dis*. 2003;36(9):1139-1143.

68. Kainulainen L, Vuorinen T, Rantakokko-Jalava K, Osterback R, Ruuskanen O. Recurrent and persistent respiratory tract viral infections in patients with primary hypogammaglobulinemia. *J Allergy Clin Immunol*. 2010;126(1):120-126.

69. Papadopoulos NG, Johnston SL. Rhinoviruses as pathogens of the lower respiratory tract. *Can Respir J*. 2000;7(5):409-414.

70. Liggett SB, Bochkov YA, Pappas T, et al. Genome sequences of rhinovirus a isolates from wisconsin pediatric respiratory studies. *Genome Announc*. 2014;2(2):10.1128/genomeA.00200-14.

71. Rahamat-Langendoen JC, Riezebos-Brilman A, Hak E, Scholvinck EH, Niesters HG. The significance of rhinovirus detection in hospitalized children: Clinical, epidemiological and virological features. *Clin Microbiol Infect*. 2013;19(10):E435-42.

72. Gerna G, Piralla A, Rovida F, et al. Correlation of rhinovirus load in the respiratory tract and clinical symptoms in hospitalized immunocompetent and immunocompromised patients. *J Med Virol*. 2009;81(8):1498-1507.

73. Piralla A, Lilleri D, Sarasini A, et al. Human rhinovirus and human respiratory enterovirus (EV68 and EV104) infections in hospitalized patients in italy, 2008-2009. *Diagn Microbiol Infect Dis*. 2012;73(2):162-167.

74. Takeyama A, Hashimoto K, Sato M, et al. Rhinovirus load and disease severity in children with lower respiratory tract infections. *J Med Virol*. 2012;84(7):1135-1142.

75. Costa C, Bergallo M, Astegiano S, et al. Detection of human rhinoviruses in the lower respiratory tract of lung transplant recipients. *Arch Virol*. 2011;156(8):1439-1443.

76. Lopez-Souza N, Dolganov G, Dubin R, et al. Resistance of differentiated human airway epithelium to infection by rhinovirus. *Am J Physiol Lung Cell Mol Physiol*. 2004;286(2):L373-81.

77. Mosser AG, Brockman-Schneider R, Amineva S, et al. Similar frequency of rhinovirus-infectible cells in upper and lower airway epithelium. *J Infect Dis*. 2002;185(6):734-743.

78. Papadopoulos NG, Bates PJ, Bardin PG, et al. Rhinoviruses infect the lower airways. *J Infect Dis*. 2000;181(6):1875-1884.

79. Lopez-Souza N, Favoreto S, Wong H, et al. In vitro susceptibility to rhinovirus infection is greater for bronchial than for nasal airway epithelial cells in human subjects. *J Allergy Clin Immunol*. 2009;123(6):1384-90.e2.

80. van der Zalm MM, van Ewijk BE, Wilbrink B, Uiterwaal CS, Wolfs TF, van der Ent CK. Respiratory pathogens in children with and without respiratory symptoms. *J Pediatr*. 2009;154(3):396-400, 400.e1.

81. Winther B, Hayden FG, Hendley JO. Picornavirus infections in children diagnosed by RT-PCR during longitudinal surveillance with weekly sampling: Association with symptomatic illness and effect of season. *J Med Virol*. 2006;78(5):644-650.

82. Fry AM, Lu X, Olsen SJ, et al. Human rhinovirus infections in rural thailand: Epidemiological evidence for rhinovirus as both pathogen and bystander. *PLoS One*. 2011;6(3):e17780.

83. Graat JM, Schouten EG, Heijnen ML, et al. A prospective, community-based study on virologic assessment among elderly people with and without symptoms of acute respiratory infection. *J Clin Epidemiol*. 2003;56(12):1218-1223.

84. Peltola V, Waris M, Osterback R, Susi P, Ruuskanen O, Hyypia T. Rhinovirus transmission within families with children: Incidence of symptomatic and asymptomatic infections. *J Infect Dis*. 2008;197(3):382-389.

85. Jartti T, Paul-Anttila M, Lehtinen P, et al. Systemic T-helper and T-regulatory cell type cytokine responses in rhinovirus vs. respiratory syncytial virus induced early wheezing: An observational study. *Respir Res*. 2009;10:85-9921-10-85.

86. Hall DJ, Bates ME, Guar L, Cronan M, Korpi N, Bertics PJ. The role of p38 MAPK in rhinovirus-induced monocyte chemoattractant protein-1 production by monocytic-lineage cells. *J Immunol*. 2005;174(12):8056-8063.

87. Khaitov MR, Laza-Stanca V, Edwards MR, et al. Respiratory virus induction of alpha-, beta- and lambda-interferons in bronchial epithelial cells and peripheral blood mononuclear cells. *Allergy*. 2009;64(3):375-386.

88. Laza-Stanca V, Stanciu LA, Message SD, Edwards MR, Gern JE, Johnston SL. Rhinovirus replication in human macrophages induces NF-kappaB-dependent tumor necrosis factor alpha production. *J Virol*. 2006;80(16):8248-8258.

89. Korpi-Steiner NL, Valkenaar SM, Bates ME, Evans MD, Gern JE, Bertics PJ. Human monocytic cells direct the robust release of CXCL10 by bronchial epithelial cells during rhinovirus infection. *Clin Exp Allergy*. 2010;40(8):1203-1213.

90. Jarjour NN, Gern JE, Kelly EA, Swenson CA, Dick CR, Busse WW. The effect of an experimental rhinovirus 16 infection on bronchial lavage neutrophils. *J Allergy Clin Immunol*. 2000;105(6 Pt 1):1169-1177.

91. Winther B, Farr B, Turner RB, Hendley JO, Gwaltney JM,Jr, Mygind N. Histopathologic examination and enumeration of polymorphonuclear leukocytes in the nasal mucosa during experimental rhinovirus colds. *Acta Otolaryngol Suppl*. 1984;413:19-24.

92. Barclay WS, al-Nakib W, Higgins PG, Tyrrell DA. The time course of the humoral immune response to rhinovirus infection. *Epidemiol Infect*. 1989;103(3):659-669.

93. Fleet WF, Couch RB, Cate TR, Knight V. Homologous and heterologous resistance to rhinovirus common cold. *Am J Epidemiol*. 1965;82(2):185-196.

94. Papi A, Contoli M. Rhinovirus vaccination: The case against. *Eur Respir J*. 2011;37(1):5-7.

95. Hall CE, Cooney MK, Fox JP. The seattle virus watch program. I. infection and illness experience of virus watch families during a communitywide epidemic of echovirus type 30 aseptic meningitis. *Am J Public Health Nations Health*. 1970;60(8):1456-1465.

96. Hendley JO, Wenzel RP, Gwaltney JM,Jr. Transmission of rhinovirus colds by self-inoculation. *N Engl J Med*. 1973;288(26):1361-1364.

97. Hicks LA, Shepard CW, Britz PH, et al. Two outbreaks of severe respiratory disease in nursing homes associated with rhinovirus. *J Am Geriatr Soc*. 2006;54(2):284-289.

98. Hall CB, Douglas RG,Jr, Geiman JM. Possible transmission by fomites of respiratory syncytial virus. *J Infect Dis*. 1980;141(1):98-102.

99. D'Alessio DJ, Meschievitz CK, Peterson JA, Dick CR, Dick EC. Short-duration exposure and the transmission of rhinoviral colds. *J Infect Dis*. 1984;150(2):189-194.

100. D'Alessio DJ, Peterson JA, Dick CR, Dick EC. Transmission of experimental rhinovirus colds in volunteer married couples. *J Infect Dis*. 1976;133(1):28-36.

101. Holmes MJ, Reed SE, Stott EJ, Tyrrell DA. Studies of experimental rhinovirus type 2 infections in polar isolation and in England. *J Hyg (Lond)*. 1976;76(3):379-393.

102. Rohde GG. Rhinovirus vaccination: The case in favour. *Eur Respir J*. 2011;37(1):3-4.

103. Thibaut HJ, De Palma AM, Neyts J. Combating enterovirus replication: State-of-the-art on antiviral research. *Biochem Pharmacol*. 2012;83(2):185-192.

104. Gwaltney JM,Jr, Park J, Paul RA, Edelman DA, O'Connor RR, Turner RB. Randomized controlled trial of clemastine fumarate for treatment of experimental rhinovirus colds. *Clin Infect Dis*. 1996;22(4):656-662.

105. Gwaltney JM,Jr, Druce HM. Efficacy of brompheniramine maleate for the treatment of rhinovirus colds. *Clin Infect Dis*. 1997;25(5):1188-1194.

106. Hayden FG, Gwaltney JM,Jr. Intranasal interferon-alpha 2 treatment of experimental rhinoviral colds. *J Infect Dis*. 1984;150(2):174-180.

107. Hayden FG, Albrecht JK, Kaiser DL, Gwaltney JM,Jr. Prevention of natural colds by contact prophylaxis with intranasal alpha 2-interferon. *N Engl J Med*. 1986;314(2):71-75.

108. Hayden FG, Kaiser DL, Albrecht JK. Intranasal recombinant alfa-2b interferon treatment of naturally occurring common colds. *Antimicrob Agents Chemother*. 1988;32(2):224-230.

109. Sutter AI, Lemiengre M, Campbell H, Mackinnon HF. Antihistamines for the common cold. *Cochrane Database Syst Rev*. 2003;(3)(3):CD001267.

110. Eccles R, Eriksson M, Garreffa S, Chen SC. The nasal decongestant effect of xylometazoline in the common cold. *Am J Rhinol*. 2008;22(5):491-496.

111. Paul IM, Beiler JS, King TS, Clapp ER, Vallati J, Berlin CM,Jr. Vapor rub, petrolatum, and no treatment for children with nocturnal cough and cold symptoms. *Pediatrics*. 2010;126(6):1092-1099.

112. Harisch G, Kretschmer M. Some aspects of a non-linear effect of zinc ions on the histamine release from rat peritoneal mast cells. *Res Commun Chem Pathol Pharmacol*. 1987;55(1):39-48.

113. Korant BD, Butterworth BE. Inhibition by zinc of rhinovirus protein cleavage: Interaction of zinc with capsid polypeptides. *J Virol*. 1976;18(1):298-306.

114. Science M, Johnstone J, Roth DE, Guyatt G, Loeb M. Zinc for the treatment of the common cold: A systematic review and meta-analysis of randomized controlled trials. *CMAJ*. 2012;184(10):E551-61.

115. Das RR, Singh M. Zinc lozenges for the common cold: Should we ignore the side-effects? *Med Hypotheses*. 2011;77(2):308-309.

116. Glass RJ, Glass LM, Beyeler WE, Min HJ. Targeted social distancing design for pandemic influenza. *Emerg Infect Dis*. 2006;12(11):1671-1681.

117. Kelso JK, Milne GJ, Kelly H. Simulation suggests that rapid activation of social distancing can arrest epidemic development due to a novel strain of influenza. *BMC Public Health*. 2009;9:117-2458-9-117.

118. Broderick MP, Hansen CJ, Russell KL. Exploration of the effectiveness of social distancing on respiratory pathogen transmission implicates environmental contributions. *J Infect Dis*. 2008;198(10):1420-1426.

119. Jefferson T, Del Mar CB, Dooley L, et al. Physical interventions to interrupt or reduce the spread of respiratory viruses. *Cochrane Database Syst Rev*. 2011;(7):CD006207. doi(7):CD006207.

120. Loeb M, Dafoe N, Mahony J, et al. Surgical mask vs N95 respirator for preventing influenza among health care workers: A randomized trial. *JAMA*. 2009;302(17):1865-1871.

121. Turner RB, Fuls JL, Rodgers ND, Goldfarb HB, Lockhart LK, Aust LB. A randomized trial of the efficacy of hand disinfection for prevention of rhinovirus infection. *Clin Infect Dis*. 2012;54(10):1422-1426.

122. Turner RB, Fuls JL, Rodgers ND. Effectiveness of hand sanitizers with and without organic acids for removal of rhinovirus from hands. *Antimicrob Agents Chemother*. 2010;54(3):1363-1364.

123. Minor PD, Muir P. Enteroviruses. In: Zuckerman AJ, Banatvala JE, Schoub BD, Griffiths PD, Mortimer P, eds. *Principles and practice of clinical virology.* 6th ed. Chichester: John Wiley & Sons, Ltd.; 2009:601-621.

124. Kogon A, Spigland I, Frothingham TE, et al. The virus watch program: A continuing surveillance of viral infections in metropolitan new york families. VII. observations on viral excretion, seroimmunity, intrafamilial spread and illness association in coxsackie and echovirus infections. *Am J Epidemiol*. 1969;89(1):51-61.

125. Horstmann DM. Enterovirus infections of the central nervous system. the present and future of poliomyelitis. *Med Clin North Am*. 1967;51(3):681-692.

126. Kono R. Apollo 11 disease or acute hemorrhagic conjunctivitis: A pandemic of a new enterovirus infection of the eyes. *Am J Epidemiol*. 1975;101(5):383-390.

127. Sawyer LA, Hershow RC, Pallansch MA, et al. An epidemic of acute hemorrhagic conjunctivitis in american samoa caused by coxsackievirus A24 variant. *Am J Epidemiol*. 1989;130(6):1187-1198.

128. Townsend TR, Bolyard EA, Yolken RH, et al. Outbreak of coxsackie A1 gastroenteritis: A complication of bone-marrow transplantation. *Lancet*. 1982;1(8276):820-823.

129. Yolken RH, Bishop CA, Townsend TR, et al. Infectious gastroenteritis in bone-marrow-transplant recipients. *N Engl J Med*. 1982;306(17):1010-1012.

130. Chen HL, Huang JY, Chu TW, et al. Expression of VP1 protein in the milk of transgenic mice: A potential oral vaccine protects against enterovirus 71 infection. *Vaccine*. 2008;26(23):2882-2889.

131. Chung YC, Ho MS, Wu JC, et al. Immunization with virus-like particles of enterovirus 71 elicits potent immune responses and protects mice against lethal challenge. *Vaccine*. 2008;26(15):1855-1862.

132. Foo DG, Alonso S, Phoon MC, Ramachandran NP, Chow VT, Poh CL. Identification of neutralizing linear epitopes from the VP1 capsid protein of enterovirus 71 using synthetic peptides. *Virus Res*. 2007;125(1):61-68.

133. Shaw ML, Palese P. Chapter 40 *orthomyxoviridae*. In: Knipe DM, Howley PM, eds. *Fields virology.* Sixth ed. Philadelphia: Wolters Kluwer/ Lippincott Williams & Wilkins; 2013:1151-1185.

134. Hay AJ, Gregory V, Douglas AR, Lin YP. The evolution of human influenza viruses. *Philos Trans R Soc Lond B Biol Sci*. 2001;356(1416):1861-1870.

135. Jones LD, Nuttall PA. Non-viraemic transmission of thogoto virus: Influence of time and distance. *Trans R Soc Trop Med Hyg*. 1989;83(5):712-714.

136. Mateo RI, Xiao SY, Lei H, DA Rosa AP, Tesh RB. Dhori virus (orthomyxoviridae: Thogotovirus) infection in mice: A model of the pathogenesis of severe orthomyxovirus infection. *Am J Trop Med Hyg*. 2007;76(4):785-790.

137. Krossoy B, Hordvik I, Nilsen F, Nylund A, Endresen C. The putative polymerase sequence of infectious salmon anemia virus suggests a new genus within the orthomyxoviridae. *J Virol*. 1999;73(3):2136-2142.

138. Presti RM, Zhao G, Beatty WL, et al. Quaranfil, johnston atoll, and lake chad viruses are novel members of the family orthomyxoviridae. *J Virol*. 2009;83(22):11599-11606.

139. Treanor J. 165: Influenza viruses, including avian influenza and swine influenza. In: Mandell GL, Bennett JE, Dolin R, eds. *Mandell, douglas, and bennett's principles and practice of infectious diseases.* Seventh ed. Philadelphia: Churchill Livingstone Elsevier; 2010:2265-2288.

140. Wright PF, Neuman G, Kawaoka Y. Chapter 41 orthomyxoviruses. In: Knipe DM, Howley PM, eds. *Fields virology.* Sixth ed. Philadelphia: Wolters Kluwer/ Lippincott Williams & Wilkins; 2013:1186-1239.

141. Buonagurio DA, Nakada S, Parvin JD, Krystal M, Palese P, Fitch WM. Evolution of human influenza A viruses over 50 years: Rapid, uniform rate of change in NS gene. *Science*. 1986;232(4753):980-982.

142. Fourment M, Wood JT, Gibbs AJ, Gibbs MJ. Evolutionary dynamics of the N1 neuraminidases of the main lineages of influenza A viruses. *Mol Phylogenet Evol*. 2010;56(2):526-535.

143. Webster RG, Bean WJ, Gorman OT, Chambers TM, Kawaoka Y. Evolution and ecology of influenza A viruses. *Microbiol Rev*. 1992;56(1):152-179.

144. Mackay IM. VDU's blog: Influenza viruses (IFVs). . 2013.

145. Biere B, Bauer B, Schweiger B. Differentiation of influenza B virus lineages yamagata and victoria by real-time PCR. *J Clin Microbiol*. 2010;48(4):1425-1427.

146. Tong S, Li Y, Rivailler P, et al. A distinct lineage of influenza A virus from bats. *Proc Natl Acad Sci U S A*. 2012;109(11):4269-4274.

147. Holsinger LJ, Nichani D, Pinto LH, Lamb RA. Influenza A virus M2 ion channel protein: A structure-function analysis. *J Virol*. 1994;68(3):1551-1563.

148. Sha B, Luo M. Structure of a bifunctional membrane-RNA binding protein, influenza virus matrix protein M1. *Nat Struct Biol*. 1997;4(3):239-244.

149. Richardson JC, Akkina RK. NS2 protein of influenza virus is found in purified virus and phosphorylated in infected cells. *Arch Virol*. 1991;116(1-4):69-80.

150. Russell CA, Jones TC, Barr IG, et al. The global circulation of seasonal influenza A (H3N2) viruses. *Science*. 2008;320(5874):340-346.

151. Dowell SF. Seasonal variation in host susceptibility and cycles of certain infectious diseases. *Emerg Infect Dis*. 2001;7(3):369-374.

152. Finkelman BS, Viboud C, Koelle K, Ferrari MJ, Bharti N, Grenfell BT. Global patterns in seasonal activity of influenza A/H3N2, A/H1N1, and B from 1997 to 2005: Viral coexistence and latitudinal gradients. *PLoS One*. 2007;2(12):e1296.

153. Lipsitch M, Viboud C. Influenza seasonality: Lifting the fog. *Proc Natl Acad Sci U S A*. 2009;106(10):3645-3646.

154. Lofgren E, Fefferman NH, Naumov YN, Gorski J, Naumova EN. Influenza seasonality: Underlying causes and modeling theories. *J Virol*. 2007;81(11):5429-5436.

155. Hemmes JH, Winkler KC, Kool SM. Virus survival as a seasonal factor in influenza and polimyelitis. *Nature*. 1960;188:430-431.

156. Hemmes JH, Winkler KC, Kool SM. Virus survival as a seasonal factor in influenza and poliomylitis. *Antonie Van Leeuwenhoek*. 1962;28:221-233.

157. Chew FT, Doraisingham S, Ling AE, Kumarasinghe G, Lee BW. Seasonal trends of viral respiratory tract infections in the tropics. *Epidemiol Infect*. 1998;121(1):121-128.

158. de Mello WA, de Paiva TM, Ishida MA, et al. The dilemma of influenza vaccine recommendations when applied to the tropics: The brazilian case examined under alternative scenarios. *PLoS One*. 2009;4(4):e5095.

159. Moura FE. Influenza in the tropics. *Curr Opin Infect Dis*. 2010;23(5):415-420.

160. Park AW, Glass K. Dynamic patterns of avian and human influenza in east and southeast asia. *Lancet Infect Dis*. 2007;7(8):543-548.

161. Viboud C, Alonso WJ, Simonsen L. Influenza in tropical regions. *PLoS Med*. 2006;3(4):e89.

162. Bedford T, Cobey S, Beerli P, Pascual M. Global migration dynamics underlie evolution and persistence of human influenza A (H3N2). *PLoS Pathog*. 2010;6(5):e1000918.

163. Chan J, Holmes A, Rabadan R. Network analysis of global influenza spread. *PLoS Comput Biol*. 2010;6(11):e1001005.

164. Nelson MI, Simonsen L, Viboud C, Miller MA, Holmes EC. Phylogenetic analysis reveals the global migration of seasonal influenza A viruses. *PLoS Pathog*. 2007;3(9):1220-1228.

165. Rambaut A, Pybus OG, Nelson MI, Viboud C, Taubenberger JK, Holmes EC. The genomic and epidemiological dynamics of human influenza A virus. *Nature*. 2008;453(7195):615-619.

166. World Health Organization. Influenza (seasonal) fact sheet no 211. http://www.who.int/mediacentre/factsheets/fs211/en/. Updated March, 2014. Accessed June/12, 2016.

167. Creanza N, Schwarz JS, Cohen JE. Intraseasonal dynamics and dominant sequences in H3N2 influenza. *PLoS One*. 2010;5(1):e8544.

168. Bush RM, Bender CA, Subbarao K, Cox NJ, Fitch WM. Predicting the evolution of human influenza A. *Science*. 1999;286(5446):1921-1925.

169. Plotkin JB, Dushoff J. Codon bias and frequency-dependent selection on the hemagglutinin epitopes of influenza A virus. *Proc Natl Acad Sci U S A*. 2003;100(12):7152-7157.

170. Reid AH, Taubenberger JK. The 1918 flu and other influenza pandemics: "Over there" and back again. *Lab Invest*. 1999;79(2):95-101.

171. Garten RJ, Davis CT, Russell CA, et al. Antigenic and genetic characteristics of swine-origin 2009 A(H1N1) influenza viruses circulating in humans. *Science*. 2009;325(5937):197-201.

172. Novel Swine-Origin Influenza A (H1N1) Virus Investigation Team, Dawood FS, Jain S, et al. Emergence of a novel swine-origin influenza A (H1N1) virus in humans. *N Engl J Med*. 2009;360(25):2605-2615.

173. Smith GJ, Vijaykrishna D, Bahl J, et al. Origins and evolutionary genomics of the 2009 swine-origin H1N1 influenza A epidemic. *Nature*. 2009;459(7250):1122-1125.

174. Trifonov V, Khiabanian H, Greenbaum B, Rabadan R. The origin of the recent swine influenza A(H1N1) virus infecting humans. *Euro Surveill*. 2009;14(17):19193.

175. Rozo M, Gronvall GK. The reemergent 1977 H1N1 strain and the gain-of-function debate. *MBio*. 2015;6(4):10.1128/mBio.01013-15.

176. Fox JP, Hall CE, Cooney MK, Foy HM. Influenzavirus infections in seattle families, 1975-1979. I. study design, methods and the occurrence of infections by time and age. *Am J Epidemiol*. 1982;116(2):212-227.

177. Glezen WP, Couch RB. Interpandemic influenza in the houston area, 1974-76. *N Engl J Med*. 1978;298(11):587-592.

178. Monto AS, Davenport FM, Napier JA, Francis T,Jr. Effect of vaccination of a school-age population upon the course of an A2-hong kong influenza epidemic. *Bull World Health Organ*. 1969;41(3):537-542.

179. Glezen WP. Serious morbidity and mortality associated with influenza epidemics. *Epidemiol Rev*. 1982;4:25-44.

180. Grijalva CG, Craig AS, Dupont WD, et al. Estimating influenza hospitalizations among children. *Emerg Infect Dis*. 2006;12(1):103-109.

181. Mullooly JP, Bridges CB, Thompson WW, et al. Influenza- and RSV-associated hospitalizations among adults. *Vaccine*. 2007;25(5):846-855.

182. Simonsen L. The global impact of influenza on morbidity and mortality. *Vaccine*. 1999;17 Suppl 1:S3-10.

183. Simonsen L, Fukuda K, Schonberger LB, Cox NJ. The impact of influenza epidemics on hospitalizations. *J Infect Dis*. 2000;181(3):831-837.

184. Fox JP, Cooney MK, Hall CE, Foy HM. Influenzavirus infections in seattle families, 1975-1979. II. pattern of infection in invaded households and relation of age and prior antibody to occurrence of infection and related illness. *Am J Epidemiol*. 1982;116(2):228-242.

185. Jordan WS,Jr, Badger GF, Dingle JH. A study of illness in a group of cleveland families. XVI. the epidemiology of influenza, 1948-1953. *Am J Hyg*. 1958;68(2):169-189.

186. Retailliau HF, Storch GA, Curtis AC, Horne TJ, Scally MJ, Hattwick MA. The epidemiology of influenza B in a rural setting in 1977. *Am J Epidemiol*. 1979;109(6):639-649.

187. Barker WH, Mullooly JP. Pneumonia and influenza deaths during epidemics: Implications for prevention. *Arch Intern Med*. 1982;142(1):85-89.

188. Barker WH, Mullooly JP. Impact of epidemic type A influenza in a defined adult population. *Am J Epidemiol*. 1980;112(6):798-811.

189. Greenberg M, Jacobziner H, Pakter J, Weisl BA. Maternal mortality in the epidemic of asian influenza, new york city, 1957. *Am J Obstet Gynecol*. 1958;76(4):897-902.

190. Neuzil KM, Reed GW, Mitchel EF, Simonsen L, Griffin MR. Impact of influenza on acute cardiopulmonary hospitalizations in pregnant women. *Am J Epidemiol*. 1998;148(11):1094-1102.

191. Barker WH, Mullooly JP. "A study of excess mortality during influenza epidemics in the united states, 1968-1976". *Am J Epidemiol*. 1982;115(3):479-480.

192. Izurieta HS, Thompson WW, Kramarz P, et al. Influenza and the rates of hospitalization for respiratory disease among infants and young children. *N Engl J Med*. 2000;342(4):232-239.

193. Sprenger MJ, Mulder PG, Beyer WE, Van Strik R, Masurel N. Impact of influenza on mortality in relation to age and underlying disease, 1967-1989. *Int J Epidemiol*. 1993;22(2):334-340.

194. Thompson WW, Weintraub E, Dhankhar P, et al. Estimates of US influenza-associated deaths made using four different methods. *Influenza Other Respir Viruses*. 2009;3(1):37-49.

195. Jordan WS,Jr. The mechanism of spread of asian influenza. *Am Rev Respir Dis*. 1961;83(2)Pt 2:29-40.

196. Taubenberger JK, Morens DM. 1918 influenza: The mother of all pandemics. *Emerg Infect Dis*. 2006;12(1):15-22.

197. Walters JH. Influenza 1918: The contemporary perspective. *Bull N Y Acad Med*. 1978;54(9):855-864.

198. Patterson KD, Pyle GF. The geography and mortality of the 1918 influenza pandemic. *Bull Hist Med*. 1991;65(1):4-21.

199. Kasowski EJ, Garten RJ, Bridges CB. Influenza pandemic epidemiologic and virologic diversity: Reminding ourselves of the possibilities. *Clin Infect Dis*. 2011;52 Suppl 1:S44-9.

200. Leung GM, Nicoll A. Reflections on pandemic (H1N1) 2009 and the international response. *PLoS Med*. 2010;7(10):10.1371/journal.pmed.1000346.

201. Schoenbaum SC. Economic impact of influenza. the individual's perspective. *Am J Med*. 1987;82(6A):26-30.

202. Molinari NA, Ortega-Sanchez IR, Messonnier ML, et al. The annual impact of seasonal influenza in the US: Measuring disease burden and costs. *Vaccine*. 2007;25(27):5086-5096.

203. World Health Organization Global Influenza Programme, ed. *A manual for estimating disease burden associated with seasonal influenza.* Geneva: World Health Organization; 2015.

204. Hinshaw VS, Webster RG, Bean WJ, Sriram G. The ecology of influenza viruses in ducks and analysis of influenza viruses with monoclonal antibodies. *Comp Immunol Microbiol Infect Dis*. 1980;3(1-2):155-164.

205. Hinshaw VS, Webster RG, Turner B. The perpetuation of orthomyxoviruses and paramyxoviruses in canadian waterfowl. *Can J Microbiol*. 1980;26(5):622-629.

206. Rohm C, Zhou N, Suss J, Mackenzie J, Webster RG. Characterization of a novel influenza hemagglutinin, H15: Criteria for determination of influenza A subtypes. *Virology*. 1996;217(2):508-516.

207. Shortridge KF. Pandemic influenza: A zoonosis? *Semin Respir Infect*. 1992;7(1):11-25.

208. Shu LP, Sharp GB, Lin YP, et al. Genetic reassortment in pandemic and interpandemic influenza viruses. A study of 122 viruses infecting humans. *Eur J Epidemiol*. 1996;12(1):63-70.

209. Banks J, Speidel E, Alexander DJ. Characterisation of an avian influenza A virus isolated from a human--is an intermediate host necessary for the emergence of pandemic influenza viruses? *Arch Virol*. 1998;143(4):781-787.

210. Claas EC, Osterhaus AD, van Beek R, et al. Human influenza A H5N1 virus related to a highly pathogenic avian influenza virus. *Lancet*. 1998;351(9101):472-477.

211. Claas EC, de Jong JC, van Beek R, Rimmelzwaan GF, Osterhaus AD. Human influenza virus A/HongKong/156/97 (H5N1) infection. *Vaccine*. 1998;16(9-10):977-978.

212. de Jong JC, Claas EC, Osterhaus AD. Influenza A (H5N1) in hong kong: Forerunner of a pandemic or just a scientifically interesting phenomenon and a useful exercise in pandemiology? *Ned Tijdschr Geneeskd*. 1998;142(22):1252-1256.

213. Guo Y, Li J, Cheng X. Discovery of men infected by avian influenza A (H9N2) virus. *Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi*. 1999;13(2):105-108.

214. Kurtz J, Manvell RJ, Banks J. Avian influenza virus isolated from a woman with conjunctivitis. *Lancet*. 1996;348(9031):901-902.

215. Lin YP, Shaw M, Gregory V, et al. Avian-to-human transmission of H9N2 subtype influenza A viruses: Relationship between H9N2 and H5N1 human isolates. *Proc Natl Acad Sci U S A*. 2000;97(17):9654-9658.

216. Peiris M, Yuen KY, Leung CW, et al. Human infection with influenza H9N2. *Lancet*. 1999;354(9182):916-917.

217. Subbarao K, Klimov A, Katz J, et al. Characterization of an avian influenza A (H5N1) virus isolated from a child with a fatal respiratory illness. *Science*. 1998;279(5349):393-396.

218. Ohishi K, Ninomiya A, Kida H, et al. Serological evidence of transmission of human influenza A and B viruses to caspian seals (phoca caspica). *Microbiol Immunol*. 2002;46(9):639-644.

219. Osterhaus AD, Rimmelzwaan GF, Martina BE, Bestebroer TM, Fouchier RA. Influenza B virus in seals. *Science*. 2000;288(5468):1051-1053.

220. Guo YJ, Jin FG, Wang P, Wang M, Zhu JM. Isolation of influenza C virus from pigs and experimental infection of pigs with influenza C virus. *J Gen Virol*. 1983;64 (Pt 1)(Pt 1):177-182.

221. Manuguerra JC, Hannoun C. Natural infection of dogs by influenza C virus. *Res Virol*. 1992;143(3):199-204.

222. Ohwada K, Kitame F, Sugawara K, Nishimura H, Homma M, Nakamura K. Distribution of the antibody to influenza C virus in dogs and pigs in yamagata prefecture, japan. *Microbiol Immunol*. 1987;31(12):1173-1180.

223. Davies JR, Grilli EA, Smith AJ. Infection with influenza A H1N1. 2. the effect of past experience on natural challenge. *J Hyg (Lond)*. 1986;96(2):345-352.

224. Frank AL, Taber LH. Variation in frequency of natural reinfection with influenza A viruses. *J Med Virol*. 1983;12(1):17-23.

225. Poehling KA, Edwards KM, Weinberg GA, et al. The underrecognized burden of influenza in young children. *N Engl J Med*. 2006;355(1):31-40.

226. Sonoguchi T, Sakoh M, Kunita N, Satsuta K, Noriki H, Fukumi H. Reinfection with influenza A (H2N2, H3N2, and H1N1) viruses in soldiers and students in japan. *J Infect Dis*. 1986;153(1):33-40.

227. Davies JR, Grilli EA, Smith AJ. Influenza A: Infection and reinfection. *J Hyg (Lond)*. 1984;92(1):125-127.

228. Price DA, Postlethwaite RJ, Longson M. Influenzavirus A2 infections presenting with febril convulsions and gastrointestinal symptoms in young children. *Clin Pediatr (Phila)*. 1976;15(4):361-367.

229. Bisno AL, Griffin JP, Van Epps KA, Niell HB, Rytel MW. Pneumonia and hong kong influenza: A prospective study of the 1968-1969 epidemic. *Am J Med Sci*. 1971;261(5):251-263.

230. Centers for Disease Control and Prevention (CDC). Severe methicillin-resistant staphylococcus aureus community-acquired pneumonia associated with influenza--louisiana and georgia, december 2006-january 2007. *MMWR Morb Mortal Wkly Rep*. 2007;56(14):325-329.

231. Ferson MJ, Morton JR, Robertson PW. Impact of influenza on morbidity in children with cystic fibrosis. *J Paediatr Child Health*. 1991;27(5):308-311.

232. Howard JB, McCracken GH,Jr, Luby JP. Influenza A 2 virus as a cause of croup requiring tracheotomy. *J Pediatr*. 1972;81(6):1148-1150.

233. Monto AS, Koopman JS, Longini IM,Jr. Tecumseh study of illness. XIII. influenza infection and disease, 1976-1981. *Am J Epidemiol*. 1985;121(6):811-822.

234. Schwarzmann SW, Adler JL, Sullivan RJ,Jr, Marine WM. Bacterial pneumonia during the hong kong influenza epidemic of 1968-1969. *Arch Intern Med*. 1971;127(6):1037-1041.

235. Crum-Cianflone NF. Bacterial, fungal, parasitic, and viral myositis. *Clin Microbiol Rev*. 2008;21(3):473-494.

236. Minow RA, Gorbach S, Johnson BL,Jr, Dornfeld L. Myoglobinuria associated with influenza A infection. *Ann Intern Med*. 1974;80(3):359-361.

237. Hurwitz ES, Nelson DB, Davis C, Morens D, Schonberger LB. National surveillance for reye syndrome: A five-year review. *Pediatrics*. 1982;70(6):895-900.

238. Rogers MF, Schonberger LB, Hurwitz ES, Rowley DL. National reye syndrome surveillance, 1982. *Pediatrics*. 1985;75(2):260-264.

239. Edelen JS, Bender TR, Chin TD. Encephalopathy and pericarditis during an outbreak of influenza. *Am J Epidemiol*. 1974;100(2):79-84.

240. Flewett TH, Hoult JG. Influenzal encephalopathy and postinfluenzal encephalitis. *Lancet*. 1958;2(7036):11-15.

241. Hayase Y, Tobita K. Influenza virus and neurological diseases. *Psychiatry Clin Neurosci*. 1997;51(4):181-184.

242. Griffiths PD, Ronalds CJ, Heath RB. A prospective study of influenza infections during pregnancy. *J Epidemiol Community Health*. 1980;34(2):124-128.

243. Hakosalo J, Saxen L. Influenza epidemic and congenital defects. *Lancet*. 1971;2(7738):1346-1347.

244. MacKenzie JS, Houghton M. Influenza infections during pregnancy: Association with congenital malformations and with subsequent neoplasms in children, and potential hazards of live virus vaccines. *Bacteriol Rev*. 1974;38(4):356-370.

245. Monif GR, Sowards DL, Eitzman DV. Serologic and immunologic evaluation of neonates following maternal influenza infection during the second and third trimesters of gestation. *Am J Obstet Gynecol*. 1972;114(2):239-242.

246. Kim HW, Brandt CD, Arrobio JO, Murphy B, Chanock RM, Parrott RH. Influenza A and B virus infection in infants and young children during the years 1957-1976. *Am J Epidemiol*. 1979;109(4):464-479.

247. Wright PF, Bryant JD, Karzon DT. Comparison of influenza B/hong kong virus infections among infants, children, and young adults. *J Infect Dis*. 1980;141(4):430-435.

248. Katayose M, Hosoya M, Haneda T, et al. The effectiveness of trivalent inactivated influenza vaccine in children over six consecutive influenza seasons. *Vaccine*. 2011;29(9):1844-1849.

249. Baine WB, Luby JP, Martin SM. Severe illness with influenza B. *Am J Med*. 1980;68(2):181-189.

250. Frank AL, Taber LH, Glezen WP, Geyer EA, McIlwain S, Paredes A. Influenza B virus infections in the community and the family. the epidemics of 1976-1977 and 1979-1980 in houston, texas. *Am J Epidemiol*. 1983;118(3):313-325.

251. Nolan TF,Jr, Goodman RA, Hinman AR, Noble GR, Kendal AP, Thacker SB. Morbidity and mortality associated with influenza B in the united states, 1979-1980. A report from the center for disease control. *J Infect Dis*. 1980;142(3):360-362.

252. Moriuchi H, Katsushima N, Nishimura H, Nakamura K, Numazaki Y. Community-acquired influenza C virus infection in children. *J Pediatr*. 1991;118(2):235-238.

253. O'Callaghan RJ, Gohd RS, Labat DD. Human antibody to influenza C virus: Its age-related distribution and distinction from receptor analogs. *Infect Immun*. 1980;30(2):500-505.

254. Matsuzaki Y, Katsushima N, Nagai Y, et al. Clinical features of influenza C virus infection in children. *J Infect Dis*. 2006;193(9):1229-1235.

255. Bucasas KL, Franco LM, Shaw CA, et al. Early patterns of gene expression correlate with the humoral immune response to influenza vaccination in humans. *J Infect Dis*. 2011;203(7):921-929.

256. Karlas A, Machuy N, Shin Y, et al. Genome-wide RNAi screen identifies human host factors crucial for influenza virus replication. *Nature*. 2010;463(7282):818-822.

257. Konig R, Stertz S, Zhou Y, et al. Human host factors required for influenza virus replication. *Nature*. 2010;463(7282):813-817.

258. Nakaya HI, Wrammert J, Lee EK, et al. Systems biology of vaccination for seasonal influenza in humans. *Nat Immunol*. 2011;12(8):786-795.

259. Nakaya HI, Li S, Pulendran B. Systems vaccinology: Learning to compute the behavior of vaccine induced immunity. *Wiley Interdiscip Rev Syst Biol Med*. 2012;4(2):193-205.

260. Shapira SD, Gat-Viks I, Shum BO, et al. A physical and regulatory map of host-influenza interactions reveals pathways in H1N1 infection. *Cell*. 2009;139(7):1255-1267.

261. Watanabe T, Watanabe S, Kawaoka Y. Cellular networks involved in the influenza virus life cycle. *Cell Host Microbe*. 2010;7(6):427-439.

262. Guillot L, Le Goffic R, Bloch S, et al. Involvement of toll-like receptor 3 in the immune response of lung epithelial cells to double-stranded RNA and influenza A virus. *J Biol Chem*. 2005;280(7):5571-5580.

263. Le Goffic R, Balloy V, Lagranderie M, et al. Detrimental contribution of the toll-like receptor (TLR)3 to influenza A virus-induced acute pneumonia. *PLoS Pathog*. 2006;2(6):e53.

264. Miettinen M, Sareneva T, Julkunen I, Matikainen S. IFNs activate toll-like receptor gene expression in viral infections. *Genes Immun*. 2001;2(6):349-355.

265. Xing Z, Harper R, Anunciacion J, et al. Host immune and apoptotic responses to avian influenza virus H9N2 in human tracheobronchial epithelial cells. *Am J Respir Cell Mol Biol*. 2011;44(1):24-33.

266. Diebold SS, Kaisho T, Hemmi H, Akira S, Reis e Sousa C. Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. *Science*. 2004;303(5663):1529-1531.

267. Geeraedts F, Goutagny N, Hornung V, et al. Superior immunogenicity of inactivated whole virus H5N1 influenza vaccine is primarily controlled by toll-like receptor signalling. *PLoS Pathog*. 2008;4(8):e1000138.

268. Lund JM, Alexopoulou L, Sato A, et al. Recognition of single-stranded RNA viruses by toll-like receptor 7. *Proc Natl Acad Sci U S A*. 2004;101(15):5598-5603.

269. Matsumoto M, Kikkawa S, Kohase M, Miyake K, Seya T. Establishment of a monoclonal antibody against human toll-like receptor 3 that blocks double-

stranded RNA-mediated signaling. *Biochem Biophys Res Commun*. 2002;293(5):1364-1369.

270. Matsumoto M, Funami K, Tanabe M, et al. Subcellular localization of toll-like receptor 3 in human dendritic cells. *J Immunol*. 2003;171(6):3154-3162.

271. Kadowaki N, Ho S, Antonenko S, et al. Subsets of human dendritic cell precursors express different toll-like receptors and respond to different microbial antigens. *J Exp Med*. 2001;194(6):863-869.

272. Le Goffic R, Pothlichet J, Vitour D, et al. Cutting edge: Influenza A virus activates TLR3-dependent inflammatory and RIG-I-dependent antiviral responses in human lung epithelial cells. *J Immunol*. 2007;178(6):3368-3372.

273. Garcia-Sastre A, Durbin RK, Zheng H, et al. The role of interferon in influenza virus tissue tropism. *J Virol*. 1998;72(11):8550-8558.

274. Koerner I, Kochs G, Kalinke U, Weiss S, Staeheli P. Protective role of beta interferon in host defense against influenza A virus. *J Virol*. 2007;81(4):2025-2030.

275. Shahangian A, Chow EK, Tian X, et al. Type I IFNs mediate development of postinfluenza bacterial pneumonia in mice. *J Clin Invest*. 2009;119(7):1910-1920.

276. Pauli EK, Schmolke M, Wolff T, et al. Influenza A virus inhibits type I IFN signaling via NF-kappaB-dependent induction of SOCS-3 expression. *PLoS Pathog*. 2008;4(11):e1000196.

277. Pothlichet J, Chignard M, Si-Tahar M. Cutting edge: Innate immune response triggered by influenza A virus is negatively regulated by SOCS1 and SOCS3 through a RIG-I/IFNAR1-dependent pathway. *J Immunol*. 2008;180(4):2034-2038.

278. Davidson S, McCabe TM, Crotta S, et al. IFNlambda is a potent anti-influenza therapeutic without the inflammatory side effects of IFNalpha treatment. *EMBO Mol Med*. 2016;8(9):1099-1112.

279. Galani IE, Triantafyllia V, Eleminiadou EE, et al. Interferon-lambda mediates non-redundant front-line antiviral protection against influenza virus infection without compromising host fitness. *Immunity*. 2017;46(5):875-890.e6.

280. Thomas PG, Keating R, Hulse-Post DJ, Doherty PC. Cell-mediated protection in influenza infection. *Emerg Infect Dis*. 2006;12(1):48-54.

281. Doherty PC, Topham DJ, Tripp RA, Cardin RD, Brooks JW, Stevenson PG. Effector CD4+ and CD8+ T-cell mechanisms in the control of respiratory virus infections. *Immunol Rev*. 1997;159:105-117.

282. Wei CJ, Boyington JC, McTamney PM, et al. Induction of broadly neutralizing H1N1 influenza antibodies by vaccination. *Science*. 2010;329(5995):1060-1064.

283. Bender BS, Croghan T, Zhang L, Small PA,Jr. Transgenic mice lacking class I major histocompatibility complex-restricted T cells have delayed viral clearance and increased mortality after influenza virus challenge. *J Exp Med*. 1992;175(4):1143-1145.

284. Eichelberger M, Allan W, Zijlstra M, Jaenisch R, Doherty PC. Clearance of influenza virus respiratory infection in mice lacking class I major histocompatibility complex-restricted CD8+ T cells. *J Exp Med*. 1991;174(4):875-880.

285. Eichelberger MC, Wang ML, Allan W, Webster RG, Doherty PC. Influenza virus RNA in the lung and lymphoid tissue of immunologically intact and CD4-depleted mice. *J Gen Virol*. 1991;72 (Pt 7)(Pt 7):1695-1698.

286. Lightman S, Cobbold S, Waldmann H, Askonas BA. Do L3T4+ T cells act as effector cells in protection against influenza virus infection. *Immunology*. 1987;62(1):139-144.

287. Riberdy JM, Christensen JP, Branum K, Doherty PC. Diminished primary and secondary influenza virus-specific CD8(+) T-cell responses in CD4-depleted ig(-/-) mice. *J Virol*. 2000;74(20):9762-9765.

288. Lazar A, Wright PF. Cell-mediated immune response of human lymphocytes to influenza A/USSR (H1N1) virus infection. *Infect Immun*. 1980;27(3):867-871.

289. Ekiert DC, Bhabha G, Elsliger MA, et al. Antibody recognition of a highly conserved influenza virus epitope. *Science*. 2009;324(5924):246-251.

290. Okuno Y, Isegawa Y, Sasao F, Ueda S. A common neutralizing epitope conserved between the hemagglutinins of influenza A virus H1 and H2 strains. *J Virol*. 1993;67(5):2552-2558.

291. Sui J, Hwang WC, Perez S, et al. Structural and functional bases for broad-spectrum neutralization of avian and human influenza A viruses. *Nat Struct Mol Biol*. 2009;16(3):265-273.

292. Karron RA, Callahan K, Luke C, et al. A live attenuated H9N2 influenza vaccine is well tolerated and immunogenic in healthy adults. *J Infect Dis*. 2009;199(5):711-716.

293. Talaat KR, Karron RA, Callahan KA, et al. A live attenuated H7N3 influenza virus vaccine is well tolerated and immunogenic in a phase I trial in healthy adults. *Vaccine*. 2009;27(28):3744-3753.

294. Voeten JT, Bestebroer TM, Nieuwkoop NJ, Fouchier RA, Osterhaus AD, Rimmelzwaan GF. Antigenic drift in the influenza A virus (H3N2) nucleoprotein and escape from recognition by cytotoxic T lymphocytes. *J Virol*. 2000;74(15):6800-6807.

295. Couch RB, Kasel JA. Immunity to influenza in man. *Annu Rev Microbiol*. 1983;37:529-549.

296. Couch RB. An overview of serum antibody responses to influenza virus antigens. *Dev Biol (Basel)*. 2003;115:25-30.

297. Gerhard W. The role of the antibody response in influenza virus infection. *Curr Top Microbiol Immunol*. 2001;260:171-190.

298. Luke TC, Kilbane EM, Jackson JL, Hoffman SL. Meta-analysis: Convalescent blood products for spanish influenza pneumonia: A future H5N1 treatment? *Ann Intern Med*. 2006;145(8):599-609.

299. Cretescu L, Beare AS, Schild GC. Formation of antibody to matrix protein in experimental human influenza A virus infections. *Infect Immun*. 1978;22(2):322-327.

300. Gerhard W. The analysis of the monoclonal immune response to influenza virus. II. the antigenicity of the viral hemagglutinin. *J Exp Med*. 1976;144(4):985-995.

301. Potter CW, Oxford JS. Determinants of immunity to influenza infection in man. *Br Med Bull*. 1979;35(1):69-75.

302. Epstein SL, Misplon JA, Lawson CM, Subbarao EK, Connors M, Murphy BR. Beta 2-microglobulin-deficient mice can be protected against influenza A infection by vaccination with vaccinia-influenza recombinants expressing hemagglutinin and neuraminidase. *J Immunol*. 1993;150(12):5484-5493.

303. Murphy BR, Clements ML. The systemic and mucosal immune response of humans to influenza A virus. *Curr Top Microbiol Immunol*. 1989;146:107-116.

304. Barbey-Martin C, Gigant B, Bizebard T, et al. An antibody that prevents the hemagglutinin low pH fusogenic transition. *Virology*. 2002;294(1):70-74.

305. Knossow M, Gaudier M, Douglas A, et al. Mechanism of neutralization of influenza virus infectivity by antibodies. *Virology*. 2002;302(2):294-298.

306. Couch RB, Kasel JA, Gerin JL, Schulman JL, Kilbourne ED. Induction of partial immunity to influenza by a neuraminidase-specific vaccine. *J Infect Dis*. 1974;129(4):411-420.

307. Kilbourne ED, Laver WG, Schulman JL, Webster RG. Antiviral activity of antiserum specific for an influenza virus neuraminidase. *J Virol*. 1968;2(4):281-288.

308. Murphy BR, Kasel JA, Chanock RM. Association of serum anti-neuraminidase antibody with resistance to influenza in man. *N Engl J Med*. 1972;286(25):1329-1332.

309. Powers DC, Kilbourne ED, Johansson BE. Neuraminidase-specific antibody responses to inactivated influenza virus vaccine in young and elderly adults. *Clin Diagn Lab Immunol*. 1996;3(5):511-516.

310. Webster RG, Reay PA, Laver WG. Protection against lethal influenza with neuraminidase. *Virology*. 1988;164(1):230-237.

311. Murphy BR, Nelson DL, Wright PF, Tierney EL, Phelan MA, Chanock RM. Secretory and systemic immunological response in children infected with live attenuated influenza A virus vaccines. *Infect Immun*. 1982;36(3):1102-1108.

312. Puleston R, Bugg G, Hoschler K, et al. Multi-centre observational study of transplacental transmission of influenza antibodies following vaccination with AS03(A)-adjuvanted H1N1 2009 vaccine. *PLoS One*. 2013;8(1):e47448.

313. Puleston RL, Bugg G, Hoschler K, et al. Observational study to investigate vertically acquired passive immunity in babies of mothers vaccinated against H1N1v during pregnancy. *Health Technol Assess*. 2010;14(55):1-82.

314. Zaman K, Roy E, Arifeen SE, et al. Effectiveness of maternal influenza immunization in mothers and infants. *N Engl J Med*. 2008;359(15):1555-1564.

315. McMichael AJ, Gotch FM, Noble GR, Beare PA. Cytotoxic T-cell immunity to influenza. *N Engl J Med*. 1983;309(1):13-17.

316. Ennis FA, Rook AH, Qi YH, et al. HLA restricted virus-specific cytotoxic T-lymphocyte responses to live and inactivated influenza vaccines. *Lancet*. 1981;2(8252):887-891.

317. Braciale TJ. Immunologic recognition of influenza virus-infected cells. I. generation of a virus-strain specific and a cross-reactive subpopulation of cytotoxic T cells in the response to type A influenza viruses of different subtypes. *Cell Immunol*. 1977;33(2):423-436.

318. Epstein SL. Prior H1N1 influenza infection and susceptibility of cleveland family study participants during the H2N2 pandemic of 1957: An experiment of nature. *J Infect Dis*. 2006;193(1):49-53.

319. Kreijtz JH, de Mutsert G, van Baalen CA, Fouchier RA, Osterhaus AD, Rimmelzwaan GF. Cross-recognition of avian H5N1 influenza virus by human cytotoxic T-lymphocyte populations directed to human influenza A virus. *J Virol*. 2008;82(11):5161-5166.

320. Zweerink HJ, Courtneidge SA, Skehel JJ, Crumpton MJ, Askonas BA. Cytotoxic T cells kill influenza virus infected cells but do not distinguish between serologically distinct type A viruses. *Nature*. 1977;267(5609):354-356.

321. Lamb JR, Eckels DD, Lake P, Johnson AH, Hartzman RJ, Woody JN. Antigen-specific human T lymphocyte clones: Induction, antigen specificity, and MHC restriction of influenza virus-immune clones. *J Immunol*. 1982;128(1):233-238.

322. Lamb JR, Woody JN, Hartzman RJ, Eckels DD. In vitro influenza virus-specific antibody production in man: Antigen-specific and HLA-restricted induction of helper activity mediated by cloned human T lymphocytes. *J Immunol*. 1982;129(4):1465-1470.

323. Lamb JR, McMichael AJ, Rothbard JB. T-cell recognition of influenza viral antigens. *Hum Immunol*. 1987;19(2):79-89.

324. Scherle PA, Gerhard W. Functional analysis of influenza-specific helper T cell clones in vivo. T cells specific for internal viral proteins provide cognate help for B cell responses to hemagglutinin. *J Exp Med*. 1986;164(4):1114-1128.

325. Caton AJ, Gerhard W. The diversity of the CD4+ T cell response in influenza. *Semin Immunol*. 1992;4(2):85-90.

326. Hashimoto G, Wright PF, Karzon DT. Antibody-dependent cell-mediated cytotoxicity against influenza virus-infected cells. *J Infect Dis*. 1983;148(5):785-794.

327. Quinnan GV, Ennis FA, Tuazon CU, et al. Cytotoxic lymphocytes and antibody-dependent complement-mediated cytotoxicity induced by administration of influenza vaccine. *Infect Immun*. 1980;30(2):362-369.

328. Ruben FL, Bachmayer H, Fireman P. Parameters of humoral and cellular immunity of influenza A/USSR/90/77 (H1N1) virus in various age groups. *J Infect Dis*. 1980;142(5):699-703.

329. Dolin R, Murphy BR, Caplan EA. Lymphocyte blastogenic responses to influenza virus antigens after influenza infection and vaccination in humans. *Infect Immun*. 1978;19(3):867-874.

330. Killingley B, Greatorex J, Cauchemez S, et al. Virus shedding and environmental deposition of novel A (H1N1) pandemic influenza virus: Interim findings. *Health Technol Assess*. 2010;14(46):237-354.

331. Killingley B, Greatorex J, Digard P, et al. The environmental deposition of influenza virus from patients infected with influenza A(H1N1)pdm09: Implications for infection prevention and control. *J Infect Public Health*. 2016;9(3):278-288.

332. Moser MR, Bender TR, Margolis HS, Noble GR, Kendal AP, Ritter DG. An outbreak of influenza aboard a commercial airliner. *Am J Epidemiol*. 1979;110(1):1-6.

333. Teunis PF, Brienen N, Kretzschmar ME. High infectivity and pathogenicity of influenza A virus via aerosol and droplet transmission. *Epidemics*. 2010;2(4):215-222.

334. Lowen AC, Steel J, Mubareka S, Palese P. High temperature (30 degrees C) blocks aerosol but not contact transmission of influenza virus. *J Virol*. 2008;82(11):5650-5652.

335. Steel J, Palese P, Lowen AC. Transmission of a 2009 pandemic influenza virus shows a sensitivity to temperature and humidity similar to that of an H3N2 seasonal strain. *J Virol*. 2011;85(3):1400-1402.

336. de Jong JC, Claas EC, Osterhaus AD, Webster RG, Lim WL. A pandemic warning? *Nature*. 1997;389(6651):554.

337. Yuen KY, Chan PK, Peiris M, et al. Clinical features and rapid viral diagnosis of human disease associated with avian influenza A H5N1 virus. *Lancet*. 1998;351(9101):467-471.

338. World Health Organization Global Influenza Programme. Cumulative number of confirmed human cases for avian influenza A(H5N1) reported to WHO, 2003-2017. http://www.who.int/influenza/human_animal_interface/2017_07_25_tableH5N1.pdf?ua=1. Updated 25 July, 2017. Accessed 22 September, 2017.

339. Fouchier RA, Schneeberger PM, Rozendaal FW, et al. Avian influenza A virus (H7N7) associated with human conjunctivitis and a fatal case of acute respiratory distress syndrome. *Proc Natl Acad Sci U S A*. 2004;101(5):1356-1361.

340. Koopmans M, Wilbrink B, Conyn M, et al. Transmission of H7N7 avian influenza A virus to human beings during a large outbreak in commercial poultry farms in the netherlands. *Lancet*. 2004;363(9409):587-593.

341. Butt KM, Smith GJ, Chen H, et al. Human infection with an avian H9N2 influenza A virus in hong kong in 2003. *J Clin Microbiol*. 2005;43(11):5760-5767.

342. Cheng VC, Chan JF, Wen X, et al. Infection of immunocompromised patients by avian H9N2 influenza A virus. *J Infect*. 2011;62(5):394-399.

343. Centers for Disease Control and Prevention (CDC). Update: Influenza activity--united states and worldwide, 2003-04 season, and composition of the 2004-05 influenza vaccine. *MMWR Morb Mortal Wkly Rep*. 2004;53(25):547-552.

344. Avian influenza A/(H7N2) outbreak in the united kingdom. *Euro Surveill*. 2007;12(5):E070531.2.

345. Hirst M, Astell CR, Griffith M, et al. Novel avian influenza H7N3 strain outbreak, british columbia. *Emerg Infect Dis*. 2004;10(12):2192-2195.

346. Tweed SA, Skowronski DM, David ST, et al. Human illness from avian influenza H7N3, british columbia. *Emerg Infect Dis*. 2004;10(12):2196-2199.

347. Vittecoq M, Grandhomme V, Champagnon J, et al. High influenza a virus infection rates in mallards bred for hunting in the camargue, south of france. *PLoS One*. 2012;7(8):e43974.

348. Nguyen-Van-Tam JS, Nair P, Acheson P, et al. Outbreak of low pathogenicity H7N3 avian influenza in UK, including associated case of human conjunctivitis. *Euro Surveill*. 2006;11(5):E060504.2.

349. World Health Organization. Background and summary of human infection with avian influenza A(H7N9) virus. http://www.who.int/influenza/human_animal_interface/20140131_background_and_summary_H7N9_v1.pdf?ua=1. Updated 31 January, 2014. Accessed 24 April, 2017.

350. World Health Organization. Analysis of recent scientific information on avian influenza A(H7N9) virus. http://www.who.int/influenza/human_animal_interface/avian_influenza/riskassessment_AH7N9_201702/en/. Updated 10 Feb., 2017. Accessed 2 April, 2017.

351. World Health Organization. Disease outbreak news: Human infection with avian influenza A(H5N6) virus - china. http://www.who.int/csr/don/08-june-2016-ah5n6-china/en/. Updated June, 2016. Accessed June/21, 2016.

352. World Health Organization. Avian influenza fact sheet. http://www.who.int/mediacentre/factsheets/avian_influenza/en/. Updated March, 2014. Accessed June/21, 2016.

353. Killingley B, Nguyen-Van-Tam J. Routes of influenza transmission. *Influenza Other Respir Viruses*. 2013;7 Suppl 2:42-51.

354. Matsuoka Y, Lamirande EW, Subbarao K. The ferret model for influenza. *Curr Protoc Microbiol*. 2009;Chapter 15:Unit 15G.2.

355. O'Donnell CD, Subbarao K. The contribution of animal models to the understanding of the host range and virulence of influenza A viruses. *Microbes Infect*. 2011;13(5):502-515.

356. Lowen AC, Mubareka S, Tumpey TM, Garcia-Sastre A, Palese P. The guinea pig as a transmission model for human influenza viruses. *Proc Natl Acad Sci U S A*. 2006;103(26):9988-9992.

357. Mubareka S, Lowen AC, Steel J, Coates AL, Garcia-Sastre A, Palese P. Transmission of influenza virus via aerosols and fomites in the guinea pig model. *J Infect Dis*. 2009;199(6):858-865.

358. Killingley B, Enstone J, Booy R, et al. Potential role of human challenge studies for investigation of influenza transmission. *Lancet Infect Dis*. 2011;11(11):879-886.

359. Cox NJ, Subbarao K. Influenza. *Lancet*. 1999;354(9186):1277-1282.

360. Cox NJ. Prevention and control of influenza. *Lancet*. 1999;354 Suppl:SIV30.

361. Ison MG, Mills J, Openshaw P, Zambon M, Osterhaus A, Hayden F. Current research on respiratory viral infections: Fourth international symposium. *Antiviral Res*. 2002;55(2):227-278.

362. Centers for Disease Control and Prevention (CDC). Licensure of a high-dose inactivated influenza vaccine for persons aged >or=65 years (fluzone high-dose) and guidance for use - united states, 2010. *MMWR Morb Mortal Wkly Rep*. 2010;59(16):485-486.

363. Fiore AE, Uyeki TM, Broder K, et al. Prevention and control of influenza with vaccines: Recommendations of the advisory committee on immunization practices (ACIP), 2010. *MMWR Recomm Rep*. 2010;59(RR-8):1-62.

364. Goodwin K, Viboud C, Simonsen L. Antibody response to influenza vaccination in the elderly: A quantitative review. *Vaccine*. 2006;24(8):1159-1169.

365. McElhaney JE. The unmet need in the elderly: Designing new influenza vaccines for older adults. *Vaccine*. 2005;23 Suppl 1:S10-25.

366. Palache AM, Beyer WE, Sprenger MJ, et al. Antibody response after influenza immunization with various vaccine doses: A double-blind, placebo-controlled, multi-centre, dose-response study in elderly nursing-home residents and young volunteers. *Vaccine*. 1993;11(1):3-9.

367. Sullivan SJ, Jacobson R, Poland GA. Advances in the vaccination of the elderly against influenza: Role of a high-dose vaccine. *Expert Rev Vaccines*. 2010;9(10):1127-1133.

368. Centers for Disease Control and Prevention (CDC). Influenza (flu) vaccination - who should do it, who should not and who should take precautions. http://www.cdc.gov/flu/protect/whoshouldvax.htm#flu-shot. Updated May, 2016. Accessed June/21, 2016.

369. Haber P, Moro PL, Lewis P, Woo EJ, Jankosky C, Cano M. Post-licensure surveillance of quadrivalent inactivated influenza (IIV4) vaccine in the united states, vaccine adverse event reporting system (VAERS), july 1, 2013-may 31, 2015. *Vaccine*. 2016;34(22):2507-2512.

370. Uhart M, Bricout H, Clay E, Largeron N. Public health and economic impact of seasonal influenza vaccination with quadrivalent influenza vaccines compared to trivalent influenza vaccines in europe. *Hum Vaccin Immunother*. 2016:1-10.

371. Impagliazzo A, Milder F, Kuipers H, et al. A stable trimeric influenza hemagglutinin stem as a broadly protective immunogen. *Science*. 2015;349(6254):1301-1306.

372. Yassine HM, Boyington JC, McTamney PM, et al. Hemagglutinin-stem nanoparticles generate heterosubtypic influenza protection. *Nat Med*. 2015;21(9):1065-1070.

373. Bright RA, Medina MJ, Xu X, et al. Incidence of adamantane resistance among influenza A (H3N2) viruses isolated worldwide from 1994 to 2005: A cause for concern. *Lancet*. 2005;366(9492):1175-1181.

374. Fiore AE, Fry A, Shay D, et al. Antiviral agents for the treatment and chemoprophylaxis of influenza --- recommendations of the advisory committee on immunization practices (ACIP). *MMWR Recomm Rep*. 2011;60(1):1-24.

375. Bridges CB, Lim W, Hu-Primmer J, et al. Risk of influenza A (H5N1) infection among poultry workers, hong kong, 1997-1998. *J Infect Dis*. 2002;185(8):1005-1010.

376. Centers for Disease Control and Prevention (CDC). Update: Isolation of avian influenza A(H5N1) viruses from humans--hong kong, 1997-1998. *MMWR Morb Mortal Wkly Rep*. 1998;46(52-53):1245-1247.

377. Govorkova EA, Leneva IA, Goloubeva OG, Bush K, Webster RG. Comparison of efficacies of RWJ-270201, zanamivir, and oseltamivir against H5N1, H9N2, and other avian influenza viruses. *Antimicrob Agents Chemother*. 2001;45(10):2723-2732.

378. Leneva IA, Roberts N, Govorkova EA, Goloubeva OG, Webster RG. The neuraminidase inhibitor GS4104 (oseltamivir phosphate) is efficacious against A/hong kong/156/97 (H5N1) and A/hong kong/1074/99 (H9N2) influenza viruses. *Antiviral Res*. 2000;48(2):101-115.

379. Yen HL, Monto AS, Webster RG, Govorkova EA. Virulence may determine the necessary duration and dosage of oseltamivir treatment for highly pathogenic A/vietnam/1203/04 influenza virus in mice. *J Infect Dis*. 2005;192(4):665-672.

380. World Health Organization. Influenza WHO rapid advice guidelines on pharmacological management of humans infected with avian influenza A(H5N1) virus. http://www.who.int/influenza/resources/documents/pharmacological_management_h5n1_05_2006/en/. Updated May, 2006. Accessed June/21, 2016.

381. Meijer A, Bosman A, van de Kamp EE, Wilbrink B, Du Ry van Beest Holle,M., Koopmans M. Measurement of antibodies to avian influenza virus A(H7N7) in humans by hemagglutination inhibition test. *J Virol Methods*. 2006;132(1-2):113-120.

382. van Kolfschooten F. Dutch veterinarian becomes first victim of avian influenza. *Lancet*. 2003;361(9367):1444.

383. Jain S, Fry AM. Peramivir: Another tool for influenza treatment? *Clin Infect Dis*. 2011;52(6):707-709.

384. Masters PS, Perlman S. Chapter 28 coronaviridae. In: Knipe DM, Howley PM, eds. *Fields virology.* Sixth ed. Philadelphia: Wolters Kluwer/ Lippincott Williams & Wilkins; 2013:825-858.

385. Becker WB, McIntosh K, Dees JH, Chanock RM. Morphogenesis of avian infectious bronchitis virus and a related human virus (strain 229E). *J Virol*. 1967;1(5):1019-1027.

386. Berry DM, Cruickshank JG, Chu HP, Wells RJ. The structure of infectious bronchitis virus. *Virology*. 1964;23:403-407.

387. Doyle LP, Hutchings LM. A transmissible gastroenteritis in pigs. *J Am Vet Med Assoc*. 1946;108:257-259.

388. Cheever FS, Daniels JB. A murine virus (JHM) causing disseminated encephalomyelitis with extensive destruction of myelin. *J Exp Med*. 1949;90(3):181-210.

389. Gledhill AW, Andrews CH. A hepatitis virus of mice. *Br J Exp Pathol*. 1951;32(6):559-568.

390. Almeida JD, Tyrrell DA. The morphology of three previously uncharacterized human respiratory viruses that grow in organ culture. *J Gen Virol*. 1967;1(2):175-178.

391. McIntosh K, Dees JH, Becker WB, Kapikian AZ, Chanock RM. Recovery in tracheal organ cultures of novel viruses from patients with respiratory disease. *Proc Natl Acad Sci U S A*. 1967;57(4):933-940.

392. Peiris JSM, Poon LLM. Chapter 21 coronaviruses and toroviruses. In: Zuckerman AJ, Banatvala JE, Schoub BD, Griffiths PD, Mortimer P, eds. *Principles and practice of clinical virology.* Sixth ed. Chichester: John Wiley & Sons, Ltd.; 2009:512-526.

393. Drosten C, Gunther S, Preiser W, et al. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. *N Engl J Med*. 2003;348(20):1967-1976.

394. Ksiazek TG, Erdman D, Goldsmith CS, et al. A novel coronavirus associated with severe acute respiratory syndrome. *N Engl J Med*. 2003;348(20):1953-1966.

395. Peiris JS. Severe acute respiratory syndrome (SARS). *J Clin Virol*. 2003;28(3):245-247.

396. van der Hoek L, Pyrc K, Jebbink MF, et al. Identification of a new human coronavirus. *Nat Med*. 2004;10(4):368-373.

397. van der Hoek L, Pyrc K, Berkhout B. Human coronavirus NL63, a new respiratory virus. *FEMS Microbiol Rev*. 2006;30(5):760-773.

398. Woo PC, Lau SK, Huang Y, Tsoi HW, Chan KH, Yuen KY. Phylogenetic and recombination analysis of coronavirus HKU1, a novel coronavirus from patients with pneumonia. *Arch Virol*. 2005;150(11):2299-2311.

399. Pollack MP, Pringle C, Madoff LC, Memish ZA. Latest outbreak news from ProMED-mail: Novel coronavirus -- middle east. *Int J Infect Dis*. 2013;17(2):e143-4.

400. de Groot RJ, Baker SC, Baric RS, et al. Middle east respiratory syndrome coronavirus (MERS-CoV): Announcement of the coronavirus study group. *J Virol*. 2013;87(14):7790-7792.

401. Park YS, Lee C, Kim KM, et al. The first case of the 2015 korean middle east respiratory syndrome outbreak. *Epidemiol Health*. 2015;37:e2015049.

402. Wise J. Two more cases of novel coronavirus are confirmed in UK. *BMJ*. 2013;346:f1030.

403. World Health Organization. Middle east respiratory syndrome coronavirus (MERS-CoV) - qatar. http://www.who.int/csr/don/21-september-2017-mers-uae/en/. Updated 21 Sept., 2017. Accessed Sept. 27, 2017.

404. Pyrc K, Dijkman R, Deng L, et al. Mosaic structure of human coronavirus NL63, one thousand years of evolution. *J Mol Biol*. 2006;364(5):964-973.

405. Woo PC, Lau SK, Yip CC, et al. Comparative analysis of 22 coronavirus HKU1 genomes reveals a novel genotype and evidence of natural recombination in coronavirus HKU1. *J Virol*. 2006;80(14):7136-7145.

406. Drazen JM. SARS--looking back over the first 100 days. *N Engl J Med*. 2003;349(4):319-320.

407. Isaacs D, Flowers D, Clarke JR, Valman HB, MacNaughton MR. Epidemiology of coronavirus respiratory infections. *Arch Dis Child*. 1983;58(7):500-503.

408. Gaunt ER, Hardie A, Claas EC, Simmonds P, Templeton KE. Epidemiology and clinical presentations of the four human coronaviruses 229E, HKU1, NL63, and OC43 detected over 3 years using a novel multiplex real-time PCR method. *J Clin Microbiol*. 2010;48(8):2940-2947.

409. Kaye HS, Dowdle WR. Seroepidemiologic survey of coronavirus (strain 229E) infections in a population of children. *Am J Epidemiol*. 1975;101(3):238-244.

410. Monto AS. Medical reviews. coronaviruses. *Yale J Biol Med*. 1974;47(4):234-251.

411. Monto AS, Lim SK. The Tecumseh study of respiratory illness. VI. frequency of and relationship between outbreaks of coronavirus infection. *J Infect Dis*. 1974;129(3):271-276.

412. Falsey AR, Walsh EE, Hayden FG. Rhinovirus and coronavirus infection-associated hospitalizations among older adults. *J Infect Dis*. 2002;185(9):1338-1341.

413. Gorse GJ, O'Connor TZ, Hall SL, Vitale JN, Nichol KL. Human coronavirus and acute respiratory illness in older adults with chronic obstructive pulmonary disease. *J Infect Dis*. 2009;199(6):847-857.

414. Gorse GJ, Donovan MM, Patel GB, Balasubramanian S, Lusk RH. Coronavirus and other respiratory illnesses comparing older with young adults. *Am J Med*. 2015;128(11):1251.e11-1251.e20.

415. Abdul-Rasool S, Fielding BC. Understanding human coronavirus HCoV-NL63. *Open Virol J*. 2010;4:76-84.

416. Pyrc K, Berkhout B, van der Hoek L. The novel human coronaviruses NL63 and HKU1. *J Virol*. 2007;81(7):3051-3057.

417. Chiu SS, Chan KH, Chu KW, et al. Human coronavirus NL63 infection and other coronavirus infections in children hospitalized with acute respiratory disease in hong kong, china. *Clin Infect Dis*. 2005;40(12):1721-1729.

418. Lau SK, Woo PC, Yip CC, et al. Coronavirus HKU1 and other coronavirus infections in hong kong. *J Clin Microbiol*. 2006;44(6):2063-2071.

419. Chan KH, Poon LL, Cheng VC, et al. Detection of SARS coronavirus in patients with suspected SARS. *Emerg Infect Dis*. 2004;10(2):294-299.

420. Leung DT, van Maren WW, Chan FK, et al. Extremely low exposure of a community to severe acute respiratory syndrome coronavirus: False seropositivity due to use of bacterially derived antigens. *J Virol*. 2006;80(18):8920-8928.

421. Guan Y, Zheng BJ, He YQ, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern china. *Science*. 2003;302(5643):276-278.

422. Mackay IM, Arden KE. MERS coronavirus: Diagnostics, epidemiology and transmission. *Virol J*. 2015;12:222-015-0439-5.

423. Mackay IM, Arden KE. Middle east respiratory syndrome: An emerging coronavirus infection tracked by the crowd. *Virus Res*. 2015;202:60-88.

424. World Health Organization. Middle east respiratory syndrome coronavirus (MERS-CoV) fact sheet. http://www.who.int/mediacentre/factsheets/mers-cov/en/. Updated 2015. Accessed April 24, 2017.

425. World Health Organization. Coronavirus infections: Disease outbreak news. http://www.who.int/csr/don/archive/disease/coronavirus_infections/en/. Updated 4 April, 2017. Accessed April 24, 2017.

426. Talbot HK, Crowe JE,Jr, Edwards KM, et al. Coronavirus infection and hospitalizations for acute respiratory illness in young children. *J Med Virol*. 2009;81(5):853-856.

427. Talbot HK, Shepherd BE, Crowe JE,Jr, et al. The pediatric burden of human coronaviruses evaluated for twenty years. *Pediatr Infect Dis J*. 2009;28(8):682-687.

428. Pohl-Koppe A, Raabe T, Siddell SG, ter Meulen V. Detection of human coronavirus 229E-specific antibodies using recombinant fusion proteins. *J Virol Methods*. 1995;55(2):175-183.

429. Bradburne AF, Bynoe ML, Tyrrell DA. Effects of a "new" human respiratory virus in volunteers. *Br Med J*. 1967;3(5568):767-769.

430. McIntosh K. Coronaviruses: A comparative review. In: Arber W, Haas R, Henle W, et al, eds. *Current topics in microbiology and immunology, series volume 63.* New York: Springer-Verlag Berlin Heidelberg; 1974:85-129. 10.1007/978-3-642-65775-7_3.

431. Gerna G, Passarani N, Battaglia M, Rondanelli EG. Human enteric coronaviruses: Antigenic relatedness to human coronavirus OC43 and possible etiologic role in viral gastroenteritis. *J Infect Dis*. 1985;151(5):796-803.

432. Kheyami AM, Nakagomi T, Nakagomi O, Getty B, Hart CA, Cunliffe NA. Detection of coronaviruses in children with acute gastroenteritis in maddina, saudi arabia. *Ann Trop Paediatr*. 2010;30(1):45-50.

433. Macnaughton MR, Davies HA. Human enteric coronaviruses. brief review. *Arch Virol*. 1981;70(4):301-313.

434. Makela MJ, Puhakka T, Ruuskanen O, et al. Viruses and bacteria in the etiology of the common cold. *J Clin Microbiol*. 1998;36(2):539-542.

435. Afzelius BA. Ultrastructure of human nasal epithelium during an episode of coronavirus infection. *Virchows Arch*. 1994;424(3):295-300.

436. El-Sahly HM, Atmar RL, Glezen WP, Greenberg SB. Spectrum of clinical illness in hospitalized patients with "common cold" virus infections. *Clin Infect Dis*. 2000;31(1):96-100.

437. Pene F, Merlat A, Vabret A, et al. Coronavirus 229E-related pneumonia in immunocompromised patients. *Clin Infect Dis*. 2003;37(7):929-932.

438. Vabret A, Mourez T, Gouarin S, Petitjean J, Freymuth F. An outbreak of coronavirus OC43 respiratory infection in normandy, france. *Clin Infect Dis*. 2003;36(8):985-989.

439. van der Hoek L, Ihorst G, Sure K, et al. Burden of disease due to human coronavirus NL63 infections and periodicity of infection. *J Clin Virol*. 2010;48(2):104-108.

440. van der Hoek L, Sure K, Ihorst G, et al. Croup is associated with the novel coronavirus NL63. *PLoS Med*. 2005;2(8):e240.

441. Dare RK, Fry AM, Chittaganpitch M, Sawanpanyalert P, Olsen SJ, Erdman DD. Human coronavirus infections in rural thailand: A comprehensive study using real-time reverse-transcription polymerase chain reaction assays. *J Infect Dis*. 2007;196(9):1321-1328.

442. Koetz A, Nilsson P, Linden M, van der Hoek L, Ripa T. Detection of human coronavirus NL63, human metapneumovirus and respiratory syncytial virus in children with respiratory tract infections in south-west sweden. *Clin Microbiol Infect*. 2006;12(11):1089-1096.

443. Peiris JS, Guan Y, Yuen KY. Severe acute respiratory syndrome. *Nat Med*. 2004;10(12 Suppl):S88-97.

444. Booth CM, Stewart TE. Severe acute respiratory syndrome and critical care medicine: The toronto experience. *Crit Care Med*. 2005;33(1 Suppl):S53-60.

445. Lee N, Hui D, Wu A, et al. A major outbreak of severe acute respiratory syndrome in hong kong. *N Engl J Med*. 2003;348(20):1986-1994.

446. Peiris JS, Chu CM, Cheng VC, et al. Clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonia: A prospective study. *Lancet*. 2003;361(9371):1767-1772.

447. Fowler RA, Lapinsky SE, Hallett D, et al. Critically ill patients with severe acute respiratory syndrome. *JAMA*. 2003;290(3):367-373.

448. Lew TW, Kwek TK, Tai D, et al. Acute respiratory distress syndrome in critically ill patients with severe acute respiratory syndrome. *JAMA*. 2003;290(3):374-380.

449. Chiang CH, Shih JF, Su WJ, Perng RP. Eight-month prospective study of 14 patients with hospital-acquired severe acute respiratory syndrome. *Mayo Clin Proc*. 2004;79(11):1372-1379.

450. Xie L, Liu Y, Fan B, et al. Dynamic changes of serum SARS-coronavirus IgG, pulmonary function and radiography in patients recovering from SARS after hospital discharge. *Respir Res*. 2005;6:5.

451. Cheng SK, Tsang JS, Ku KH, Wong CW, Ng YK. Psychiatric complications in patients with severe acute respiratory syndrome (SARS) during the acute treatment phase: A series of 10 cases. *Br J Psychiatry*. 2004;184:359-360.

452. Gu J, Gong E, Zhang B, et al. Multiple organ infection and the pathogenesis of SARS. *J Exp Med*. 2005;202(3):415-424.

453. Lau KK, Yu WC, Chu CM, Lau ST, Sheng B, Yuen KY. Possible central nervous system infection by SARS coronavirus. *Emerg Infect Dis*. 2004;10(2):342-344.

454. Lee DT, Wing YK, Leung HC, et al. Factors associated with psychosis among patients with severe acute respiratory syndrome: A case-control study. *Clin Infect Dis*. 2004;39(8):1247-1249.

455. Chang WT, Kao CL, Chung MY, et al. SARS exposure and emergency department workers. *Emerg Infect Dis*. 2004;10(6):1117-1119.

456. Ho KY, Singh KS, Habib AG, et al. Mild illness associated with severe acute respiratory syndrome coronavirus infection: Lessons from a prospective seroepidemiologic study of health-care workers in a teaching hospital in singapore. *J Infect Dis*. 2004;189(4):642-647.

457. Rainer TH, Chan PK, Ip M, et al. The spectrum of severe acute respiratory syndrome-associated coronavirus infection. *Ann Intern Med*. 2004;140(8):614-619.

458. Omrani AS, Matin MA, Haddad Q, Al-Nakhli D, Memish ZA, Albarrak AM. A family cluster of middle east respiratory syndrome coronavirus infections related to a likely unrecognized asymptomatic or mild case. *Int J Infect Dis*. 2013;17(9):e668-72.

459. Oboho IK, Tomczyk SM, Al-Asmari AM, et al. 2014 MERS-CoV outbreak in jeddah--a link to health care facilities. *N Engl J Med*. 2015;372(9):846-854.

460. Kraaij-Dirkzwager M, Timen A, Dirksen K, et al. Middle east respiratory syndrome coronavirus (MERS-CoV) infections in two returning travellers in the netherlands, may 2014. *Euro Surveill*. 2014;19(21):20817.

461. Mailles A, Blanckaert K, Chaud P, et al. First cases of middle east respiratory syndrome coronavirus (MERS-CoV) infections in france, investigations and implications for the prevention of human-to-human transmission, france, may 2013. *Euro Surveill*. 2013;18(24):20502.

462. Assiri A, Al-Tawfiq JA, Al-Rabeeah AA, et al. Epidemiological, demographic, and clinical characteristics of 47 cases of middle east respiratory syndrome

coronavirus disease from saudi arabia: A descriptive study. *Lancet Infect Dis*. 2013;13(9):752-761.

463. Memish ZA, Zumla AI, Al-Hakeem RF, Al-Rabeeah AA, Stephens GM. Family cluster of middle east respiratory syndrome coronavirus infections. *N Engl J Med*. 2013;368(26):2487-2494.

464. Memish ZA, Cotten M, Watson SJ, et al. Community case clusters of middle east respiratory syndrome coronavirus in hafr al-batin, kingdom of saudi arabia: A descriptive genomic study. *Int J Infect Dis*. 2014;23:63-68.

465. Cameron MJ, Ran L, Xu L, et al. Interferon-mediated immunopathological events are associated with atypical innate and adaptive immune responses in patients with severe acute respiratory syndrome. *J Virol*. 2007;81(16):8692-8706.

466. Cervantes-Barragan L, Zust R, Weber F, et al. Control of coronavirus infection through plasmacytoid dendritic-cell-derived type I interferon. *Blood*. 2007;109(3):1131-1137.

467. Roth-Cross JK, Bender SJ, Weiss SR. Murine coronavirus mouse hepatitis virus is recognized by MDA5 and induces type I interferon in brain macrophages/microglia. *J Virol*. 2008;82(20):9829-9838.

468. Zhou H, Zhao J, Perlman S. Autocrine interferon priming in macrophages but not dendritic cells results in enhanced cytokine and chemokine production after coronavirus infection. *MBio*. 2010;1(4):10.1128/mBio.00219-10.

469. Ireland DD, Stohlman SA, Hinton DR, Atkinson R, Bergmann CC. Type I interferons are essential in controlling neurotropic coronavirus infection irrespective of functional CD8 T cells. *J Virol*. 2008;82(1):300-310.

470. Ireland DD, Stohlman SA, Hinton DR, et al. RNase L mediated protection from virus induced demyelination. *PLoS Pathog*. 2009;5(10):e1000602.

471. Zhou J, Marten NW, Bergmann CC, Macklin WB, Hinton DR, Stohlman SA. Expression of matrix metalloproteinases and their tissue inhibitor during viral encephalitis. *J Virol*. 2005;79(8):4764-4773.

472. Wu GF, Perlman S. Macrophage infiltration, but not apoptosis, is correlated with immune-mediated demyelination following murine infection with a neurotropic coronavirus. *J Virol*. 1999;73(10):8771-8780.

473. Zhao J, Zhao J, Van Rooijen N, Perlman S. Evasion by stealth: Inefficient immune activation underlies poor T cell response and severe disease in SARS-CoV-infected mice. *PLoS Pathog*. 2009;5(10):e1000636.

474. Chen H, Hou J, Jiang X, et al. Response of memory CD8+ T cells to severe acute respiratory syndrome (SARS) coronavirus in recovered SARS patients and healthy individuals. *J Immunol*. 2005;175(1):591-598.

475. Liu J, Sun Y, Qi J, et al. The membrane protein of severe acute respiratory syndrome coronavirus acts as a dominant immunogen revealed by a clustering region of novel functionally and structurally defined cytotoxic T-lymphocyte epitopes. *J Infect Dis*. 2010;202(8):1171-1180.

476. Peng H, Yang LT, Wang LY, et al. Long-lived memory T lymphocyte responses against SARS coronavirus nucleocapsid protein in SARS-recovered patients. *Virology*. 2006;351(2):466-475.

477. Perlman S. Pathogenesis of coronavirus-induced infections. review of pathological and immunological aspects. *Adv Exp Med Biol*. 1998;440:503-513.

478. Lin MT, Hinton DR, Marten NW, Bergmann CC, Stohlman SA. Antibody prevents virus reactivation within the central nervous system. *J Immunol*. 1999;162(12):7358-7368.

479. Lin MT, Hinton DR, Parra B, Stohlman SA, van der Veen RC. The role of IL-10 in mouse hepatitis virus-induced demyelinating encephalomyelitis. *Virology*. 1998;245(2):270-280.

480. Trandem K, Anghelina D, Zhao J, Perlman S. Regulatory T cells inhibit T cell proliferation and decrease demyelination in mice chronically infected with a coronavirus. *J Immunol*. 2010;184(8):4391-4400.

481. Trandem K, Zhao J, Fleming E, Perlman S. Highly activated cytotoxic CD8 T cells express protective IL-10 at the peak of coronavirus-induced encephalitis. *J Immunol*. 2011;186(6):3642-3652.

482. Lipsitch M, Cohen T, Cooper B, et al. Transmission dynamics and control of severe acute respiratory syndrome. *Science*. 2003;300(5627):1966-1970.

483. Pitzer VE, Leung GM, Lipsitch M. Estimating variability in the transmission of severe acute respiratory syndrome to household contacts in hong kong, china. *Am J Epidemiol*. 2007;166(3):355-363.

484. Riley S, Fraser C, Donnelly CA, et al. Transmission dynamics of the etiological agent of SARS in hong kong: Impact of public health interventions. *Science*. 2003;300(5627):1961-1966.

485. Ho PL, Tang XP, Seto WH. SARS: Hospital infection control and admission strategies. *Respirology*. 2003;8 Suppl:S41-5.

486. Peiris JS, Guan Y. Confronting SARS: A view from hong kong. *Philos Trans R Soc Lond B Biol Sci*. 2004;359(1447):1075-1079.

487. Seto WH, Tsang D, Yung RW, et al. Effectiveness of precautions against droplets and contact in prevention of nosocomial transmission of severe acute respiratory syndrome (SARS). *Lancet*. 2003;361(9368):1519-1520.

488. Yu IT, Li Y, Wong TW, et al. Evidence of airborne transmission of the severe acute respiratory syndrome virus. *N Engl J Med*. 2004;350(17):1731-1739.

489. Rabenau HF, Kampf G, Cinatl J, Doerr HW. Efficacy of various disinfectants against SARS coronavirus. *J Hosp Infect*. 2005;61(2):107-111.

490. Rabenau HF, Cinatl J, Morgenstern B, Bauer G, Preiser W, Doerr HW. Stability and inactivation of SARS coronavirus. *Med Microbiol Immunol*. 2005;194(1-2):1-6.

491. World Health Organization. Emergencies preparedness, response: Frequently asked questions on middle east respiratory syndrome coronavirus (MERS-CoV). http://www.who.int/csr/disease/coronavirus_infections/faq/en/. Updated 12 July, 2015. Accessed May/ 25, 2016.

492. Azhar EI, El-Kafrawy SA, Farraj SA, et al. Evidence for camel-to-human transmission of MERS coronavirus. *N Engl J Med*. 2014;370(26):2499-2505.

493. Madani TA, Azhar EI, Hashem AM. Evidence for camel-to-human transmission of MERS coronavirus. *N Engl J Med*. 2014;371(14):1360.

494. Gossner C, Danielson N, Gervelmeyer A, et al. Human-dromedary camel interactions and the risk of acquiring zoonotic middle east respiratory syndrome coronavirus infection. *Zoonoses Public Health*. 2016;63(1):1-9.

495. Cauchemez S, Fraser C, Van Kerkhove MD, et al. Middle east respiratory syndrome coronavirus: Quantification of the extent of the epidemic, surveillance biases, and transmissibility. *Lancet Infect Dis*. 2014;14(1):50-56.

496. Drosten C, Muth D, Corman VM, et al. An observational, laboratory-based study of outbreaks of middle east respiratory syndrome coronavirus in jeddah and riyadh, kingdom of saudi arabia, 2014. *Clin Infect Dis*. 2015;60(3):369-377.

497. Memish ZA, Assiri AM, Al-Tawfiq JA. Middle east respiratory syndrome coronavirus (MERS-CoV) viral shedding in the respiratory tract: An observational analysis with infection control implications. *Int J Infect Dis*. 2014;29:307-308.

498. Puzelli S, Azzi A, Santini MG, et al. Investigation of an imported case of middle east respiratory syndrome coronavirus (MERS-CoV) infection in florence, italy, may to june 2013. *Euro Surveill*. 2013;18(34):20564.

499. Drosten C, Meyer B, Muller MA, et al. Transmission of MERS-coronavirus in household contacts. *N Engl J Med*. 2014;371(9):828-835.

500. Health Protection Agency (HPA) UK Novel Coronavirus Investigation team. Evidence of person-to-person transmission within a family cluster of novel coronavirus infections, united kingdom, february 2013. *Euro Surveill*. 2013;18(11):20427.

501. WHO MERS-CoV Research Group. State of knowledge and data gaps of middle east respiratory syndrome coronavirus (MERS-CoV) in humans. *PLoS Curr*. 2013;5:10.1371/currents.outbreaks.0bf719e352e7478f8ad85fa30127ddb8.

502. Anderson LJ, Tong S. Update on SARS research and other possibly zoonotic coronaviruses. *Int J Antimicrob Agents*. 2010;36 Suppl 1:S21-5.

503. Roper RL, Rehm KE. SARS vaccines: Where are we? *Expert Rev Vaccines*. 2009;8(7):887-898.

504. Saif LJ. Animal coronavirus vaccines: Lessons for SARS. *Dev Biol (Basel)*. 2004;119:129-140.

505. Jackwood MW, Boynton TO, Hilt DA, et al. Emergence of a group 3 coronavirus through recombination. *Virology*. 2010;398(1):98-108.

506. Wang L, Junker D, Collisson EW. Evidence of natural recombination within the S1 gene of infectious bronchitis virus. *Virology*. 1993;192(2):710-716.

507. Vennema H, de Groot RJ, Harbour DA, et al. Early death after feline infectious peritonitis virus challenge due to recombinant vaccinia virus immunization. *J Virol*. 1990;64(3):1407-1409.

508. de Haan CA, Volders H, Koetzner CA, Masters PS, Rottier PJ. Coronaviruses maintain viability despite dramatic rearrangements of the strictly conserved genome organization. *J Virol*. 2002;76(24):12491-12502.

509. DeDiego ML, Alvarez E, Almazan F, et al. A severe acute respiratory syndrome coronavirus that lacks the E gene is attenuated in vitro and in vivo. *J Virol*. 2007;81(4):1701-1713.

510. Yount B, Roberts RS, Lindesmith L, Baric RS. Rewiring the severe acute respiratory syndrome coronavirus (SARS-CoV) transcription circuit: Engineering a recombination-resistant genome. *Proc Natl Acad Sci U S A*. 2006;103(33):12546-12551.

511. Zust R, Cervantes-Barragan L, Kuri T, et al. Coronavirus non-structural protein 1 is a major pathogenicity factor: Implications for the rational design of coronavirus vaccines. *PLoS Pathog*. 2007;3(8):e109.

512. Stockman LJ, Bellamy R, Garner P. SARS: Systematic review of treatment effects. *PLoS Med*. 2006;3(9):e343.

513. Chan KS, Lai ST, Chu CM, et al. Treatment of severe acute respiratory syndrome with lopinavir/ritonavir: A multicentre retrospective matched cohort study. *Hong Kong Med J*. 2003;9(6):399-406.

514. Cheng Y, Wong R, Soo YO, et al. Use of convalescent plasma therapy in SARS patients in hong kong. *Eur J Clin Microbiol Infect Dis*. 2005;24(1):44-46.

515. Chu CM, Cheng VC, Hung IF, et al. Role of lopinavir/ritonavir in the treatment of SARS: Initial virological and clinical findings. *Thorax*. 2004;59(3):252-256.

516. Loutfy MR, Blatt LM, Siminovitch KA, et al. Interferon alfacon-1 plus corticosteroids in severe acute respiratory syndrome: A preliminary study. *JAMA*. 2003;290(24):3222-3228.

517. Soo YO, Cheng Y, Wong R, et al. Retrospective comparison of convalescent plasma with continuing high-dose methylprednisolone treatment in SARS patients. *Clin Microbiol Infect*. 2004;10(7):676-678.

518. Yeh KM, Chiueh TS, Siu LK, et al. Experience of using convalescent plasma for severe acute respiratory syndrome among healthcare workers in a taiwan hospital. *J Antimicrob Chemother*. 2005;56(5):919-922.

519. Liang G, Chen Q, Xu J, et al. Laboratory diagnosis of four recent sporadic cases of community-acquired SARS, guangdong province, china. *Emerg Infect Dis*. 2004;10(10):1774-1781.

520. Lim PL, Kurup A, Gopalakrishna G, et al. Laboratory-acquired severe acute respiratory syndrome. *N Engl J Med*. 2004;350(17):1740-1745.

521. Berk AJ. Chapter 55 adenoviridae. In: Knipe DM, Howley PM, eds. *Fields virology.* Sixth ed. Philadelphia: Wolters Kluwer/ Lippincott Williams & Wilkins; 2013:1704-1731.

522. Wold WSM, Ison MG. Chapter 56 adenoviruses. In: Knipe DM, Howley PM, eds. *Fields virology.* Sixth ed. Philadelphia: Wolters Kluwer/ Lippincott Williams & Wilkins; 2013:1732-1767.

523. Robinson CM, Singh G, Lee JY, et al. Molecular evolution of human adenoviruses. *Sci Rep*. 2013;3:1812.

524. Echavarria M. Chapter 19 adenoviruses. In: Zuckerman AJ, Banatvala JE, Schoub BD, Griffiths PD, Mortimer P, eds. *Principles and practice of clinical virology.* Sixth ed. Chichester: John Wiley & Sons, Ltd.; 2009:463-483.

525. Hilleman MR, Werner JH. Recovery of new agent from patients with acute respiratory illness. *Proc Soc Exp Biol Med*. 1954;85(1):183-188.

526. Rowe WP, Huebner RJ, Gilmore LK, Parrott RH, Ward TG. Isolation of a cytopathogenic agent from human adenoids undergoing spontaneous degeneration in tissue culture. *Proc Soc Exp Biol Med*. 1953;84(3):570-573.

527. Enders JF, Bell JA, Dingle JH, et al. Adenoviruses: Group name proposed for new respiratory-tract viruses. *Science*. 1956;124(3212):119-120.

528. Gray GC. Adenovirus vaccines. In: Plotkin SA, Orenstein WA, Offit PA, eds. *Vaccines.* 6th ed. Philadelphia: Elsevier; 2013:113-126.

529. Berget SM, Moore C, Sharp PA. Spliced segments at the 5' terminus of adenovirus 2 late mRNA. *Proc Natl Acad Sci U S A*. 1977;74(8):3171-3175.

530. Chow LT, Gelinas RE, Broker TR, Roberts RJ. An amazing sequence arrangement at the 5' ends of adenovirus 2 messenger RNA. *Cell*. 1977;12(1):1-8.

531. Fox JP, Brandt CD, Wassermann FE, et al. The virus watch program: A continuing surveillance of viral infections in metropolitan new york families. VI. observations of adenovirus infections: Virus excretion patterns, antibody response, efficiency of surveillance, patterns of infections, and relation to illness. *Am J Epidemiol*. 1969;89(1):25-50.

532. Fox JP, Hall CE, Cooney MK. The seattle virus watch. VII. observations of adenovirus infections. *Am J Epidemiol*. 1977;105(4):362-386.

533. Kolavic-Gray SA, Binn LN, Sanchez JL, et al. Large epidemic of adenovirus type 4 infection among military trainees: Epidemiological, clinical, and laboratory studies. *Clin Infect Dis*. 2002;35(7):808-818.

534. Cooper RJ, Hallett R, Tullo AB, Klapper PE. The epidemiology of adenovirus infections in greater manchester, UK 1982-96. *Epidemiol Infect*. 2000;125(2):333-345.

535. Gray GC, Goswami PR, Malasig MD, et al. Adult adenovirus infections: Loss of orphaned vaccines precipitates military respiratory disease epidemics. for the adenovirus surveillance group. *Clin Infect Dis*. 2000;31(3):663-670.

536. Hilleman MR. Epidemiology of adenovirus respiratory infections in military recruit populations. *Ann N Y Acad Sci*. 1957;67(8):262-272.

537. Hilleman MR. Acute respiratory illness caused by adenoviruses; a military problem. *U S Armed Forces Med J*. 1956;7(12):1717-1725.

538. Purkayastha A, Ditty SE, Su J, et al. Genomic and bioinformatics analysis of HAdV-4, a human adenovirus causing acute respiratory disease: Implications for gene therapy and vaccine vector development. *J Virol*. 2005;79(4):2559-2572.

539. Purkayastha A, Su J, Carlisle S, Tibbetts C, Seto D. Genomic and bioinformatics analysis of HAdV-7, a human adenovirus of species B1 that causes acute respiratory disease: Implications for vector development in human gene therapy. *Virology*. 2005;332(1):114-129.

540. Purkayastha A, Su J, McGraw J, et al. Genomic and bioinformatics analyses of HAdV-4vac and HAdV-7vac, two human adenovirus (HAdV) strains that constituted original prophylaxis against HAdV-related acute respiratory disease, a reemerging epidemic disease. *J Clin Microbiol*. 2005;43(7):3083-3094.

541. Rowe WP, Hartley JW, Huebner RJ. Additional serotypes of the APC virus group. *Proc Soc Exp Biol Med*. 1956;91(2):260-262.

542. Robinson CM, Singh G, Henquell C, et al. Computational analysis and identification of an emergent human adenovirus pathogen implicated in a respiratory fatality. *Virology*. 2011;409(2):141-147.

543. Walsh MP, Seto J, Jones MS, Chodosh J, Xu W, Seto D. Computational analysis identifies human adenovirus type 55 as a re-emergent acute respiratory disease pathogen. *J Clin Microbiol*. 2010;48(3):991-993.

544. Turner M, Istre GR, Beauchamp H, Baum M, Arnold S. Community outbreak of adenovirus type 7a infections associated with a swimming pool. *South Med J*. 1987;80(6):712-715.

545. Keenlyside RA, Hierholzer JC, D'Angelo LJ. Keratoconjunctivitis associated with adenovirus type 37: An extended outbreak in an ophthalmologist's office. *J Infect Dis*. 1983;147(2):191-198.

546. Filho EP, da Costa Faria NR, Fialho AM, et al. Adenoviruses associated with acute gastroenteritis in hospitalized and community children up to 5 years old in rio de janeiro and salvador, brazil. *J Med Microbiol*. 2007;56(Pt 3):313-319.

547. Yolken RH, Lawrence F, Leister F, Takiff HE, Strauss SE. Gastroenteritis associated with enteric type adenovirus in hospitalized infants. *J Pediatr*. 1982;101(1):21-26.

548. Kajon AE, Mistchenko AS, Videla C, Hortal M, Wadell G, Avendano LF. Molecular epidemiology of adenovirus acute lower respiratory infections of children in the south cone of south america (1991-1994). *J Med Virol*. 1996;48(2):151-156.

549. Kajon AE, Wadell G. Sequence analysis of the E3 region and fiber gene of human adenovirus genome type 7h. *Virology*. 1996;215(2):190-196.

550. Kajon AE, Hang J, Hawksworth A, et al. Molecular epidemiology of adenovirus type 21 respiratory strains isolated from US military trainees (1996-2014). *J Infect Dis*. 2015;212(6):871-880.

551. Niemann TH, Trigg ME, Winick N, Penick GD. Disseminated adenoviral infection presenting as acute pancreatitis. *Hum Pathol*. 1993;24(10):1145-1148.

552. Pavia AT. Viral infections of the lower respiratory tract: Old viruses, new viruses, and the role of diagnosis. *Clin Infect Dis*. 2011;52 Suppl 4:S284-9.

553. Chuang Y, Chiu CH, Wong KS, et al. Severe adenovirus infection in children. *J Microbiol Immunol Infect*. 2003;36(1):37-40.

554. Faden H, Wynn RJ, Campagna L, Ryan RM. Outbreak of adenovirus type 30 in a neonatal intensive care unit. *J Pediatr*. 2005;146(4):523-527.

555. Frabasile S, Vitureira N, Perez G, Mateos S, Arbiza J. Genotyping of uruguayan human adenovirus isolates collected between 1994 and 1998. *Acta Virol*. 2005;49(2):129-132.

556. Murtagh P, Cerqueiro C, Halac A, Avila M, Kajon A. Adenovirus type 7h respiratory infections: A report of 29 cases of acute lower respiratory disease. *Acta Paediatr*. 1993;82(6-7):557-561.

557. Metzgar D, Osuna M, Kajon AE, Hawksworth AW, Irvine M, Russell KL. Abrupt emergence of diverse species B adenoviruses at US military recruit training centers. *J Infect Dis*. 2007;196(10):1465-1473.

558. Houng HS, Lott L, Gong H, Kuschner RA, Lynch JA, Metzgar D. Adenovirus microsatellite reveals dynamics of transmission during a recent epidemic of human adenovirus serotype 14 infection. *J Clin Microbiol*. 2009;47(7):2243-2248.

559. Lewis PF, Schmidt MA, Lu X, et al. A community-based outbreak of severe respiratory illness caused by human adenovirus serotype 14. *J Infect Dis*. 2009;199(10):1427-1434.

560. Seto J, Walsh MP, Mahadevan P, et al. Genomic and bioinformatics analyses of HAdV-14p, reference strain of a re-emerging respiratory pathogen and analysis of B1/B2. *Virus Res*. 2009;143(1):94-105.

561. Tate JE, Bunning ML, Lott L, et al. Outbreak of severe respiratory disease associated with emergent human adenovirus serotype 14 at a US air force training facility in 2007. *J Infect Dis*. 2009;199(10):1419-1426.

562. Gray GC, McCarthy T, Lebeck MG, et al. Genotype prevalence and risk factors for severe clinical adenovirus infection, united states 2004-2006. *Clin Infect Dis*. 2007;45(9):1120-1131.

563. Neville JS, Bunning M, Lyons A, et al. Challenges associated with the emergence of adenovirus type 14 at US military training centers. *Mil Med*. 2008;173(7):iv-vii.

564. Sturdy PM, Court SD, Gardner PS. Viruses and whooping-cough. *Lancet*. 1971;2(7731):978-979.

565. Collier AM, Connor JD, Irving WR,Jr. Generalized type 5 adenovirus infection associated with the pertussis syndrome. *J Pediatr*. 1966;69(6):1073-1078.

566. Ferrer A, Calico I, Manresa JM, Andreu A, Moraga F, Valle I. Microorganisms isolated in cases of pertussis-like syndrome. *Enferm Infecc Microbiol Clin*. 2000;18(9):433-438.

567. Nelson KE, Gavitt F, Batt MD, Kallick CA, Reddi KT, Levin S. The role of adenoviruses in the pertussis syndrome. *J Pediatr*. 1975;86(3):335-341.

568. Reina J, Ferres F, Gutierrez O, Ruiz de Gopegui E, Gonzalez-Cardenas M. Study of the clinical and epidemiological characteristics of respiratory infections caused by adenovirus in a pediatric population (1997-2003). *An Pediatr (Barc)*. 2004;61(2):137-142.

569. Neumann R, Genersch E, Eggers HJ. Detection of adenovirus nucleic acid sequences in human tonsils in the absence of infectious virus. *Virus Res*. 1987;7(1):93-97.

570. Johansson SM, Arnberg N, Elofsson M, Wadell G, Kihlberg J. Multivalent HSA conjugates of 3'-sialyllactose are potent inhibitors of adenoviral cell attachment and infection. *Chembiochem*. 2005;6(2):358-364.

571. Nilsson EC, Storm RJ, Bauer J, et al. The GD1a glycan is a cellular receptor for adenoviruses causing epidemic keratoconjunctivitis. *Nat Med*. 2011;17(1):105-109.

572. Harvey SA, Romanowski EG, Yates KA, Gordon YJ. Adenovirus-directed ocular innate immunity: The role of conjunctival defensin-like chemokines (IP-10, I-TAC) and phagocytic human defensin-alpha. *Invest Ophthalmol Vis Sci*. 2005;46(10):3657-3665.

573. Smith JG, Nemerow GR. Mechanism of adenovirus neutralization by human alpha-defensins. *Cell Host Microbe*. 2008;3(1):11-19.

574. Smith JG, Silvestry M, Lindert S, Lu W, Nemerow GR, Stewart PL. Insight into the mechanisms of adenovirus capsid disassembly from studies of defensin neutralization. *PLoS Pathog*. 2010;6(6):e1000959.

575. Liu Q, Muruve DA. Molecular basis of the inflammatory response to adenovirus vectors. *Gene Ther*. 2003;10(11):935-940.

576. Nazir SA, Metcalf JP. Innate immune response to adenovirus. *J Investig Med*. 2005;53(6):292-304.

577. Haveman LM, de Jager W, van Loon AM, Claas EC, Prakken BJ, Bierings M. Different cytokine signatures in children with localized and invasive adenovirus infection after stem cell transplantation. *Pediatr Transplant*. 2010;14(4):520-528.

578. Feuchtinger T, Richard C, Joachim S, et al. Clinical grade generation of hexon-specific T cells for adoptive T-cell transfer as a treatment of adenovirus infection after allogeneic stem cell transplantation. *J Immunother*. 2008;31(2):199-206.

579. Heemskerk B, Lankester AC, van Vreeswijk T, et al. Immune reconstitution and clearance of human adenovirus viremia in pediatric stem-cell recipients. *J Infect Dis*. 2005;191(4):520-530.

580. Flomenberg P, Piaskowski V, Truitt RL, Casper JT. Characterization of human proliferative T cell responses to adenovirus. *J Infect Dis*. 1995;171(5):1090-1096.

581. Zandvliet ML, Falkenburg JH, van Liempt E, et al. Combined CD8+ and CD4+ adenovirus hexon-specific T cells associated with viral clearance after stem cell transplantation as treatment for adenovirus infection. *Haematologica*. 2010;95(11):1943-1951.

582. Collins RH,Jr, Goldstein S, Giralt S, et al. Donor leukocyte infusions in acute lymphocytic leukemia. *Bone Marrow Transplant*. 2000;26(5):511-516.

583. Leen AM, Christin A, Khalil M, et al. Identification of hexon-specific CD4 and CD8 T-cell epitopes for vaccine and immunotherapy. *J Virol*. 2008;82(1):546-554.

584. Baum SG. Adenovirus. In: Mandell GL, Bennet JE, Dolin R, eds. *Principles and practice of infectious diseases.* Sixth ed. Philadelphia: Churchill Livingstone; 2004.

585. Couch RB, Cate TR, Douglas RG,Jr, Gerone PJ, Knight V. Effect of route of inoculation on experimental respiratory viral disease in volunteers and evidence for airborne transmission. *Bacteriol Rev*. 1966;30(3):517-529.

586. Nauheim RC, Romanowski EG, Araullo-Cruz T, et al. Prolonged recoverability of desiccated adenovirus type 19 from various surfaces. *Ophthalmology*. 1990;97(11):1450-1453.

587. Rutala WA, Peacock JE, Gergen MF, Sobsey MD, Weber DJ. Efficacy of hospital germicides against adenovirus 8, a common cause of epidemic keratoconjunctivitis in health care facilities. *Antimicrob Agents Chemother*. 2006;50(4):1419-1424.

588. Lyons A, Longfield J, Kuschner R, et al. A double-blind, placebo-controlled study of the safety and immunogenicity of live, oral type 4 and type 7 adenovirus vaccines in adults. *Vaccine*. 2008;26(23):2890-2898.

589. Couch RB, Chanock RM, Cate TR, Lang DJ, Knight V, Huebner RJ. Immunization with types 4 and 7 adenovirus by selective infection of the intestinal tract. *Am Rev Respir Dis*. 1963;88:SUPPL 394-403.

590. Russell KL, Hawksworth AW, Ryan MA, et al. Vaccine-preventable adenoviral respiratory illness in US military recruits, 1999-2004. *Vaccine*. 2006;24(15):2835-2842.

591. Mueller RE, Muldoon RL, Jackson GG. Communicability of enteric live adenovirus type 4 vaccine in families. *J Infect Dis*. 1969;119(1):60-66.

592. Stanley ED, Jackson GG. Spread of enteric live adenovirus type 4 vaccine in married couples. *J Infect Dis*. 1969;119(1):51-59.

593. Arav-Boger R, Echavarria M, Forman M, Charache P, Persaud D. Clearance of adenoviral hepatitis with ribavirin therapy in a pediatric liver transplant recipient. *Pediatr Infect Dis J*. 2000;19(11):1097-1100.

594. Bordigoni P, Carret AS, Venard V, Witz F, Le Faou A. Treatment of adenovirus infections in patients undergoing allogeneic hematopoietic stem cell transplantation. *Clin Infect Dis*. 2001;32(9):1290-1297.

595. Hoffman JA, Shah AJ, Ross LA, Kapoor N. Adenoviral infections and a prospective trial of cidofovir in pediatric hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant*. 2001;7(7):388-394.

596. Morfin F, Dupuis-Girod S, Mundweiler S, et al. In vitro susceptibility of adenovirus to antiviral drugs is species-dependent. *Antivir Ther*. 2005;10(2):225-229.

597. Chakrabarti S, Collingham KE, Fegan CD, Pillay D, Milligan DW. Adenovirus infections following haematopoietic cell transplantation: Is there a role for adoptive immunotherapy? *Bone Marrow Transplant*. 2000;26(3):305-307.

598. Hromas R, Cornetta K, Srour E, Blanke C, Broun ER. Donor leukocyte infusion as therapy of life-threatening adenoviral infections after T-cell-depleted bone marrow transplantation. *Blood*. 1994;84(5):1689-1690.

599. Gordon YJ, Gordon RY, Romanowski E, Araullo-Cruz TP. Prolonged recovery of desiccated adenoviral serotypes 5, 8, and 19 from plastic and metal surfaces in vitro. *Ophthalmology*. 1993;100(12):1835-9; discussion 1839-40.

600. Romanowski EG, Bartels SP, Vogel R, et al. Feasibility of an antiviral clinical trial requiring cross-country shipment of conjunctival adenovirus cultures and recovery of infectious virus. *Curr Eye Res*. 2004;29(2-3):195-199.

601. Ko G, Cromeans TL, Sobsey MD. UV inactivation of adenovirus type 41 measured by cell culture mRNA RT-PCR. *Water Res*. 2005;39(15):3643-3649.

602. Nwachuku N, Gerba CP, Oswald A, Mashadi FD. Comparative inactivation of adenovirus serotypes by UV light disinfection. *Appl Environ Microbiol*. 2005;71(9):5633-5636.

603. Thurston-Enriquez JA, Haas CN, Jacangelo J, Gerba CP. Inactivation of enteric adenovirus and feline calicivirus by chlorine dioxide. *Appl Environ Microbiol*. 2005;71(6):3100-3105.

604. Thurston-Enriquez JA, Haas CN, Jacangelo J, Gerba CP. Inactivation of enteric adenovirus and feline calicivirus by ozone. *Water Res*. 2005;39(15):3650-3656.

605. Callahan SM, Wonganan P, Obenauer-Kutner LJ, Sutjipto S, Dekker JD, Croyle MA. Controlled inactivation of recombinant viruses with vitamin B2. *J Virol Methods*. 2008;148(1-2):132-145.

606. Lin L, Hanson CV, Alter HJ, et al. Inactivation of viruses in platelet concentrates by photochemical treatment with amotosalen and long-wavelength ultraviolet light. *Transfusion*. 2005;45(4):580-590.

607. Mufti NA, Erickson AC, North AK, et al. Treatment of whole blood (WB) and red blood cells (RBC) with S-303 inactivates pathogens and retains in vitro quality of stored RBC. *Biologicals*. 2010;38(1):14-19.

608. Singh Y, Sawyer LS, Pinkoski LS, et al. Photochemical treatment of plasma with amotosalen and long-wavelength ultraviolet light inactivates pathogens while retaining coagulation function. *Transfusion*. 2006;46(7):1168-1177.

609. Lamb RA, Parks GD. Chapter 33 *paramyxoviridae*. In: Knipe DM, Howley PM, eds. *Fields virology.* Sixth ed. Philadelphia: Wolters Kluwer/ Lippincot Williams & Wilkins; 2013:957-995.

610. Blount RE,Jr, Morris JA, Savage RE. Recovery of cytopathogenic agent from chimpanzees with coryza. *Proc Soc Exp Biol Med*. 1956;92(3):544-549.

611. Collins PL, Karron RA. Chapter 38 respiratory syncytial virus and metapneumovirus. In: Knipe DM, Howley PM, eds. *Fields virology.* Sixth ed. Philadelphia: Wolters Kluwer/ Lippincott Williams & Wilkins; 2013:1086-1123.

612. Falsey AR, Walsh EE. Respiratory syncytial virus infection in adults. *Clin Microbiol Rev*. 2000;13(3):371-384.

613. Englund JA, Piedra PA, Jewell A, Patel K, Baxter BB, Whimbey E. Rapid diagnosis of respiratory syncytial virus infections in immunocompromised adults. *J Clin Microbiol*. 1996;34(7):1649-1653.

614. Falsey AR, Hennessey PA, Formica MA, Cox C, Walsh EE. Respiratory syncytial virus infection in elderly and high-risk adults. *N Engl J Med*. 2005;352(17):1749-1759.

615. Walsh EE, McConnochie KM, Long CE, Hall CB. Severity of respiratory syncytial virus infection is related to virus strain. *J Infect Dis*. 1997;175(4):814-820.

616. Zambon MC, Stockton JD, Clewley JP, Fleming DM. Contribution of influenza and respiratory syncytial virus to community cases of influenza-like illness: An observational study. *Lancet*. 2001;358(9291):1410-1416.

617. Mlinaric-Galinovic G, Varda-Brkic D. Nosocomial respiratory syncytial virus infections in children's wards. *Diagn Microbiol Infect Dis*. 2000;37(4):237-246.

618. Anderson LJ, Hendry RM, Pierik LT, Tsou C, McIntosh K. Multicenter study of strains of respiratory syncytial virus. *J Infect Dis*. 1991;163(4):687-692.

619. Hall CB, Walsh EE, Schnabel KC, et al. Occurrence of groups A and B of respiratory syncytial virus over 15 years: Associated epidemiologic and clinical characteristics in hospitalized and ambulatory children. *J Infect Dis*. 1990;162(6):1283-1290.

620. Halstead DC, Jenkins SG. Continuous non-seasonal epidemic of respiratory syncytial virus infection in the southeast united states. *South Med J*. 1998;91(5):433-436.

621. Yusuf S, Piedimonte G, Auais A, et al. The relationship of meteorological conditions to the epidemic activity of respiratory syncytial virus. *Epidemiol Infect*. 2007;135(7):1077-1090.

622. Hall CB. Respiratory syncytial virus in young children. *Lancet*. 2010;375(9725):1500-1502.

623. Nair H, Nokes DJ, Gessner BD, et al. Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: A systematic review and meta-analysis. *Lancet*. 2010;375(9725):1545-1555.

624. Hall CB, Weinberg GA, Iwane MK, et al. The burden of respiratory syncytial virus infection in young children. *N Engl J Med*. 2009;360(6):588-598.

625. Lee MS, Walker RE, Mendelman PM. Medical burden of respiratory syncytial virus and parainfluenza virus type 3 infection among US children. implications for design of vaccine trials. *Hum Vaccin*. 2005;1(1):6-11.

626. Glezen WP, Taber LH, Frank AL, Kasel JA. Risk of primary infection and reinfection with respiratory syncytial virus. *Am J Dis Child*. 1986;140(6):543-546.

627. Henderson FW, Collier AM, Clyde WA,Jr, Denny FW. Respiratory-syncytial-virus infections, reinfections and immunity. A prospective, longitudinal study in young children. *N Engl J Med*. 1979;300(10):530-534.

628. Kim HW, Arrobio JO, Brandt CD, et al. Epidemiology of respiratory syncytial virus infection in washington, D.C. I. importance of the virus in different respiratory tract disease syndromes and temporal distribution of infection. *Am J Epidemiol*. 1973;98(3):216-225.

629. Boyce TG, Mellen BG, Mitchel EF,Jr, Wright PF, Griffin MR. Rates of hospitalization for respiratory syncytial virus infection among children in medicaid. *J Pediatr*. 2000;137(6):865-870.

630. Berkovich S. Acute respiratory illness in the premature nursery associated with respiratory syncytial virus infections. *Pediatrics*. 1964;34:753-760.

631. Cunningham CK, McMillan JA, Gross SJ. Rehospitalization for respiratory illness in infants of less than 32 weeks' gestation. *Pediatrics*. 1991;88(3):527-532.

632. Fishaut M, Tubergen D, McIntosh K. Cellular response to respiratory viruses with particular reference to children with disorders of cell-mediated immunity. *J Pediatr*. 1980;96(2):179-186.

633. Groothuis J, Bauman J, Malinoski F, Eggleston M. Strategies for prevention of RSV nosocomial infection. *J Perinatol*. 2008;28(5):319-323.

634. MacDonald NE, Hall CB, Suffin SC, Alexson C, Harris PJ, Manning JA. Respiratory syncytial viral infection in infants with congenital heart disease. *N Engl J Med*. 1982;307(7):397-400.

635. Thompson WW, Shay DK, Weintraub E, et al. Mortality associated with influenza and respiratory syncytial virus in the united states. *JAMA*. 2003;289(2):179-186.

636. Whimbey E, Champlin RE, Couch RB, et al. Community respiratory virus infections among hospitalized adult bone marrow transplant recipients. *Clin Infect Dis*. 1996;22(5):778-782.

637. Harrington RD, Hooton TM, Hackman RC, et al. An outbreak of respiratory syncytial virus in a bone marrow transplant center. *J Infect Dis*. 1992;165(6):987-993.

638. Whimbey E, Bodey GP. Viral pneumonia in the immunocompromised adult with neoplastic disease: The role of common community respiratory viruses. *Semin Respir Infect*. 1992;7(2):122-131.

639. Miron D, Srugo I, Kra-Oz Z, et al. Sole pathogen in acute bronchiolitis: Is there a role for other organisms apart from respiratory syncytial virus? *Pediatr Infect Dis J*. 2010;29(1):e7-e10.

640. Wolf DG, Greenberg D, Kalkstein D, et al. Comparison of human metapneumovirus, respiratory syncytial virus and influenza A virus lower respiratory tract infections in hospitalized young children. *Pediatr Infect Dis J*. 2006;25(4):320-324.

641. Murata Y, Falsey AR. Respiratory syncytial virus infection in adults. *Antivir Ther*. 2007;12(4 Pt B):659-670.

642. Hall CB, Walsh EE, Long CE, Schnabel KC. Immunity to and frequency of reinfection with respiratory syncytial virus. *J Infect Dis*. 1991;163(4):693-698.

643. Shapiro JM, Jean RE. Respiratory syncytial virus. *N Engl J Med*. 2001;345(15):1132-1133.

644. Hall CB. Respiratory syncytial virus: Its transmission in the hospital environment. *Yale J Biol Med*. 1982;55(3-4):219-223.

645. Hall CB. Nosocomial respiratory syncytial virus infections: The "cold war" has not ended. *Clin Infect Dis*. 2000;31(2):590-596.

646. Cubie HA, Duncan LA, Marshall LA, Smith NM. Detection of respiratory syncytial virus nucleic acid in archival postmortem tissue from infants. *Pediatr Pathol Lab Med*. 1997;17(6):927-938.

647. Hall CB, Kopelman AE, Douglas RG,Jr, Geiman JM, Meagher MP. Neonatal respiratory syncytial virus infection. *N Engl J Med*. 1979;300(8):393-396.

648. Tobias JD. Caffeine in the treatment of apnea associated with respiratory syncytial virus infection in neonates and infants. *South Med J*. 2000;93(3):294-296.

649. Hall CB. Respiratory syncytial virus and sudden infant death. *N Engl J Med*. 1979;300(25):1440-1441.

650. Viral infections in infancy - ERS. http://www.erswhitebook.org/chapters/early-life-events/viral-infections-in-infancy/. Accessed 2/29/2016, 2016.

651. Hall CB, Long CE, Schnabel KC. Respiratory syncytial virus infections in previously healthy working adults. *Clin Infect Dis*. 2001;33(6):792-796.

652. Tsutsumi H, Onuma M, Nagai K, Yamazaki H, Chiba S. Clinical characteristics of respiratory syncytial virus (RSV) subgroup infections in japan. *Scand J Infect Dis*. 1991;23(6):671-674.

653. Kaul TN, Welliver RC, Wong DT, Udwadia RA, Riddlesberger K, Ogra PL. Secretory antibody response to respiratory syncytial virus infection. *Am J Dis Child*. 1981;135(11):1013-1016.

654. McIntosh K, Masters HB, Orr I, Chao RK, Barkin RM. The immunologic response to infection with respiratory syncytial virus in infants. *J Infect Dis*. 1978;138(1):24-32.

655. Glezen WP, Paredes A, Allison JE, Taber LH, Frank AL. Risk of respiratory syncytial virus infection for infants from low-income families in relationship to age, sex, ethnic group, and maternal antibody level. *J Pediatr*. 1981;98(5):708-715.

656. Welliver RC, Kaul TN, Putnam TI, Sun M, Riddlesberger K, Ogra PL. The antibody response to primary and secondary infection with respiratory syncytial virus: Kinetics of class-specific responses. *J Pediatr*. 1980;96(5):808-813.

657. Falsey AR, Singh HK, Walsh EE. Serum antibody decay in adults following natural respiratory syncytial virus infection. *J Med Virol*. 2006;78(11):1493-1497.

658. Macartney KK, Gorelick MH, Manning ML, Hodinka RL, Bell LM. Nosocomial respiratory syncytial virus infections: The cost-effectiveness and cost-benefit of infection control. *Pediatrics*. 2000;106(3):520-526.

659. Falsey AR, Walsh EE. Relationship of serum antibody to risk of respiratory syncytial virus infection in elderly adults. *J Infect Dis*. 1998;177(2):463-466.

660. Mills J5, Van Kirk JE, Wright PF, Chanock RM. Experimental respiratory syncytial virus infection of adults. possible mechanisms of resistance to infection and illness. *J Immunol*. 1971;107(1):123-130.

661. Walsh EE, Falsey AR. Age related differences in humoral immune response to respiratory syncytial virus infection in adults. *J Med Virol*. 2004;73(2):295-299.

662. Walsh EE, Peterson DR, Falsey AR. Risk factors for severe respiratory syncytial virus infection in elderly persons. *J Infect Dis*. 2004;189(2):233-238.

663. Openshaw PJ. Immunity and immunopathology to respiratory syncytial virus. the mouse model. *Am J Respir Crit Care Med*. 1995;152(4 Pt 2):S59-62.

664. Openshaw PJ. Immunopathological mechanisms in respiratory syncytial virus disease. *Springer Semin Immunopathol*. 1995;17(2-3):187-201.

665. Heidema J, Lukens MV, van Maren WW, et al. CD8+ T cell responses in bronchoalveolar lavage fluid and peripheral blood mononuclear cells of infants with severe primary respiratory syncytial virus infections. *J Immunol*. 2007;179(12):8410-8417.

666. Heidema J, Rossen JW, Lukens MV, et al. Dynamics of human respiratory virus-specific CD8+ T cell responses in blood and airways during episodes of common cold. *J Immunol*. 2008;181(8):5551-5559.

667. Everard ML, Swarbrick A, Wrightham M, et al. Analysis of cells obtained by bronchial lavage of infants with respiratory syncytial virus infection. *Arch Dis Child*. 1994;71(5):428-432.

668. McNamara PS, Ritson P, Selby A, Hart CA, Smyth RL. Bronchoalveolar lavage cellularity in infants with severe respiratory syncytial virus bronchiolitis. *Arch Dis Child*. 2003;88(10):922-926.

669. Johnson JE, Gonzales RA, Olson SJ, Wright PF, Graham BS. The histopathology of fatal untreated human respiratory syncytial virus infection. *Mod Pathol*. 2007;20(1):108-119.

670. Welliver TP, Garofalo RP, Hosakote Y, et al. Severe human lower respiratory tract illness caused by respiratory syncytial virus and influenza virus is characterized by the absence of pulmonary cytotoxic lymphocyte responses. *J Infect Dis*. 2007;195(8):1126-1136.

671. Rechsteiner J. Inactivation of respiratory syncytial virus in air. *Antonie Van Leeuwenhoek*. 1969;35(2):238.

672. Bont L. Nosocomial RSV infection control and outbreak management. *Paediatr Respir Rev*. 2009;10 Suppl 1:16-17.

673. World Health Organization. WHO consultation on respiratory syncytial virus (RSV) vaccine development. http://www.who.int/immunization/research/meetings_workshops/rsv_vaccine_development/en/. Accessed 2/22/2016, 2016.

674. World Health Organization. WHO product development for vaccines advisory committee (PD-VAC) meeting – 2015. http://who.int/immunization/research/meetings_workshops/pdvac/en/. Accessed 2/22/2016, 2016.

675. McKay E, Higgins P, Tyrrell D, Pringle C. Immunogenicity and pathogenicity of temperature-sensitive modified respiratory syncytial virus in adult volunteers. *J Med Virol*. 1988;25(4):411-421.

676. Pringle CR, Filipiuk AH, Robinson BS, Watt PJ, Higgins P, Tyrrell DA. Immunogenicity and pathogenicity of a triple temperature-sensitive modified respiratory syncytial virus in adult volunteers. *Vaccine*. 1993;11(4):473-478.

677. Watt PJ, Robinson BS, Pringle CR, Tyrrell DA. Determinants of susceptibility to challenge and the antibody response of adult volunteers given experimental respiratory syncytial virus vaccines. *Vaccine*. 1990;8(3):231-236.

678. Gonzalez IM, Karron RA, Eichelberger M, et al. Evaluation of the live attenuated cpts 248/404 RSV vaccine in combination with a subunit RSV vaccine (PFP-2) in healthy young and older adults. *Vaccine*. 2000;18(17):1763-1772.

679. Rodriguez WJ, Kim HW, Brandt CD, et al. Aerosolized ribavirin in the treatment of patients with respiratory syncytial virus disease. *Pediatr Infect Dis J*. 1987;6(2):159-163.

680. Rodriguez WJ, Bui RH, Connor JD, et al. Environmental exposure of primary care personnel to ribavirin aerosol when supervising treatment of infants with respiratory syncytial virus infections. *Antimicrob Agents Chemother*. 1987;31(7):1143-1146.

681. Rodriguez WJ, Parrott RH. Ribavirin aerosol treatment of serious respiratory syncytial virus infection in infants. *Infect Dis Clin North Am*. 1987;1(2):425-439.

682. Hu J, Robinson JL. Treatment of respiratory syncytial virus with palivizumab: A systematic review. *World J Pediatr*. 2010;6(4):296-300.

683. Lagos R, DeVincenzo JP, Munoz A, et al. Safety and antiviral activity of motavizumab, a respiratory syncytial virus (RSV)-specific humanized monoclonal antibody, when administered to RSV-infected children. *Pediatr Infect Dis J*. 2009;28(9):835-837.

684. Malley R, DeVincenzo J, Ramilo O, et al. Reduction of respiratory syncytial virus (RSV) in tracheal aspirates in intubated infants by use of humanized monoclonal antibody to RSV F protein. *J Infect Dis*. 1998;178(6):1555-1561.

685. Walsh EE, Falsey AR, Hennessey PA. Respiratory syncytial and other virus infections in persons with chronic cardiopulmonary disease. *Am J Respir Crit Care Med*. 1999;160(3):791-795.

686. Whimbey E, Couch RB, Englund JA, et al. Respiratory syncytial virus pneumonia in hospitalized adult patients with leukemia. *Clin Infect Dis*. 1995;21(2):376-379.

687. Herfst S, de Graaf M, Schickli JH, et al. Recovery of human metapneumovirus genetic lineages a and B from cloned cDNA. *J Virol*. 2004;78(15):8264-8270.

688. van den Hoogen BG, de Jong JC, Groen J, et al. A newly discovered human pneumovirus isolated from young children with respiratory tract disease. *Nat Med*. 2001;7(6):719-724.

689. Aberle JH, Aberle SW, Redlberger-Fritz M, Sandhofer MJ, Popow-Kraupp T. Human metapneumovirus subgroup changes and seasonality during epidemics. *Pediatr Infect Dis J*. 2010;29(11):1016-1018.

690. Falsey AR. Human metapneumovirus infection in adults. *Pediatr Infect Dis J*. 2008;27(10 Suppl):S80-3.

691. Walsh EE, Peterson DR, Falsey AR. Human metapneumovirus infections in adults: Another piece of the puzzle. *Arch Intern Med*. 2008;168(22):2489-2496.

692. Falsey AR, Erdman D, Anderson LJ, Walsh EE. Human metapneumovirus infections in young and elderly adults. *J Infect Dis*. 2003;187(5):785-790.

693. Andrewes CH, Bang FB, Chanock RM, Zhdanov VM. Para-influenza viruses 1, 2, and 3: Suggested names for recently described myxoviruses. *Virology*. 1959;8(1):129-130.

694. Karron RA, Collins PL. Chapter 34 parainfluenza viruses. In: Knipe DM, Howley PM, eds. *Fields virology.* Sixth ed. Philadelphia: Wolters Kluwer/ Lippincott Williams & Wilkins; 2013:996-1023.

695. Komada H, Tsurudome M, Ueda M, Nishio M, Bando H, Ito Y. Isolation and characterization of monoclonal antibodies to human parainfluenza virus type 4 and their use in revealing antigenic relation between subtypes 4A and 4B. *Virology*. 1989;171(1):28-37.

696. Fairchok MP, Martin ET, Kuypers J, Englund JA. A prospective study of parainfluenza virus type 4 infections in children attending daycare. *Pediatr Infect Dis J*. 2011;30(8):714-716.

697. Hasman H, Pachucki CT, Unal A, et al. Aetiology of influenza-like illness in adults includes parainfluenzavirus type 4. *J Med Microbiol*. 2009;58(Pt 4):408-413.

698. Ren L, Gonzalez R, Xie Z, et al. Human parainfluenza virus type 4 infection in chinese children with lower respiratory tract infections: A comparison study. *J Clin Virol*. 2011;51(3):209-212.

699. Weinberg GA, Hall CB, Iwane MK, et al. Parainfluenza virus infection of young children: Estimates of the population-based burden of hospitalization. *J Pediatr*. 2009;154(5):694-699.

700. Knott AM, Long CE, Hall CB. Parainfluenza viral infections in pediatric outpatients: Seasonal patterns and clinical characteristics. *Pediatr Infect Dis J*. 1994;13(4):269-273.

701. Psarras S, Papadopoulos NG, Johnston SL. Chapter 17 parainfluenza VIruses. In: Zuckerman AJ, Banatvala JE, Schoub BD, Griffiths PD, Mortimer P, eds. *Principles and practice of clinical virology.* Sixth ed. Chichester: John Wiley & Sons, Ltd.; 2009:409-439.

702. Laurichesse H, Dedman D, Watson JM, Zambon MC. Epidemiological features of parainfluenza virus infections: Laboratory surveillance in England and Wales, 1975-1997. *Eur J Epidemiol*. 1999;15(5):475-484.

703. Glezen WP, Frank AL, Taber LH, Kasel JA. Parainfluenza virus type 3: Seasonality and risk of infection and reinfection in young children. *J Infect Dis*. 1984;150(6):851-857.

704. Parrott RH, Vargosko A, Luckey A, Kim HW, Cumming C, Chanock R. Clinical features of infection with hemadsorption viruses. *N Engl J Med*. 1959;260(15):731-738.

705. Parrott RH, Vargosko AJ, Kim H, Bell JA, Chanock RM. Acute respiratory diseases of viral etiology. III. parainfluenza. myxoviruses. *Am J Public Health Nations Health*. 1962;52:907-917.

706. Chanock RM, Parrott RH. Acute respiratory disease in infancy and childhood: Present understanding and prospects for prevention. *Pediatrics*. 1965;36:21-39.

707. Denny FW, Murphy TF, Clyde WA,Jr, Collier AM, Henderson FW. Croup: An 11-year study in a pediatric practice. *Pediatrics*. 1983;71(6):871-876.

708. Chanock RM, Parrott RH, Johnson KM, Kapikian AZ, Bell JA. Myxoviruses: Parainfluenza. *Am Rev Respir Dis*. 1963;88:SUPPL 152-66.

709. Glezen P, Denny FW. Epidemiology of acute lower respiratory disease in children. *N Engl J Med*. 1973;288(10):498-505.

710. Slavin KA, Passaro DJ, Hacker JK, Hendry RM, Kohl S. Parainfluenza virus type 4: Case report and review of the literature. *Pediatr Infect Dis J*. 2000;19(9):893-896.

711. Marx A, Gary HE,Jr, Marston BJ, et al. Parainfluenza virus infection among adults hospitalized for lower respiratory tract infection. *Clin Infect Dis*. 1999;29(1):134-140.

712. Woo PC, Young K, Tsang KW, Ooi CG, Peiris M, Yuen K. Adult croup: A rare but more severe condition. *Respiration*. 2000;67(6):684-688.

713. Fry AM, Curns AT, Harbour K, Hutwagner L, Holman RC, Anderson LJ. Seasonal trends of human parainfluenza viral infections: United states, 1990-2004. *Clin Infect Dis*. 2006;43(8):1016-1022.

714. Hall CB. Respiratory syncytial virus and parainfluenza virus. *N Engl J Med*. 2001;344(25):1917-1928.

715. Segal AO, Crighton EJ, Moineddin R, Mamdani M, Upshur RE. Croup hospitalizations in ontario: A 14-year time-series analysis. *Pediatrics*. 2005;116(1):51-55.

716. Hotez PJ, Goldstein B, Ziegler J, Doveikis SA, Pasternack MS. Adult respiratory distress syndrome associated with parainfluenza virus type 1 in children. *Pediatr Infect Dis J*. 1990;9(10):750-752.

717. Madden JF, Burchette JL,Jr, Hale LP. Pathology of parainfluenza virus infection in patients with congenital immunodeficiency syndromes. *Hum Pathol*. 2004;35(5):594-603.

718. Nichols WG, Erdman DD, Han A, Zukerman C, Corey L, Boeckh M. Prolonged outbreak of human parainfluenza virus 3 infection in a stem cell transplant outpatient department: Insights from molecular epidemiologic analysis. *Biol Blood Marrow Transplant*. 2004;10(1):58-64.

719. Arguedas A, Stutman HR, Blanding JG. Parainfluenza type 3 meningitis. report of two cases and review of the literature. *Clin Pediatr (Phila)*. 1990;29(3):175-178.

720. Arisoy ES, Demmler GJ, Thakar S, Doerr C. Meningitis due to parainfluenza virus type 3: Report of two cases and review. *Clin Infect Dis*. 1993;17(6):995-997.

721. Powell HC, Rosenberg RN, McKellar B. Reye's syndrome: Isolation of parainfluenza virus. report of three cases. *Arch Neurol*. 1973;29(3):135-139.

722. Roman G, Phillips CA, Poser CM. Parainfluenza virus type 3: Isolation from CSF of a patient with guillain-barre syndrome. *JAMA*. 1978;240(15):1613-1615.

723. Vreede RW, Schellekens H, Zuijderwijk M. Isolation of parainfluenza virus type 3 from cerebrospinal fluid. *J Infect Dis*. 1992;165(6):1166.

724. Kasel JA, Frank AL, Keitel WA, Taber LH, Glezen WP. Acquisition of serum antibodies to specific viral glycoproteins of parainfluenza virus 3 in children. *J Virol*. 1984;52(3):828-832.

725. Smith CB, Purcell RH, Bellanti JA, Chanock RM. Protective effect of antibody to parainfluenza type 1 virus. *N Engl J Med*. 1966;275(21):1145-1152.

726. Tremonti LP, Lin JS, Jackson GG. Neutralizing activity in nasal secretions and serum in resistance of volunteers to parainfluenza virus type 2. *J Immunol*. 1968;101(3):572-577.

727. Moscona A, Peluso RW. Persistent infection with human parainfluenza virus 3 in CV-1 cells: Analysis of the role of defective interfering particles. *Virology*. 1993;194(1):399-402.

728. Welliver RC, Wong DT, Middleton E,Jr, Sun M, McCarthy N, Ogra PL. Role of parainfluenza virus-specific IgE in pathogenesis of croup and wheezing subsequent to infection. *J Pediatr*. 1982;101(6):889-896.

729. Henrickson KJ. Parainfluenza viruses. *Clin Microbiol Rev*. 2003;16(2):242-264.

730. Karron RA, O'Brien KL, Froehlich JL, Brown VA. Molecular epidemiology of a parainfluenza type 3 virus outbreak on a pediatric ward. *J Infect Dis*. 1993;167(6):1441-1445.

731. Singh-Naz N, Willy M, Riggs N. Outbreak of parainfluenza virus type 3 in a neonatal nursery. *Pediatr Infect Dis J*. 1990;9(1):31-33.

732. Gross PA, Green RH, Curnen MG. Persistent infection with parainfluenza type 3 virus in man. *Am Rev Respir Dis*. 1973;108(4):894-898.

733. Crowe JE,Jr. Influence of maternal antibodies on neonatal immunization against respiratory viruses. *Clin Infect Dis*. 2001;33(10):1720-1727.

734. Karron RA, Makhene M, Gay K, Wilson MH, Clements ML, Murphy BR. Evaluation of a live attenuated bovine parainfluenza type 3 vaccine in two- to six-month-old infants. *Pediatr Infect Dis J*. 1996;15(8):650-654.

735. Leicestershire County Council. Census 2011 second release - key statistics, january. . 2012:16/06/2015.

736. Nicholson KG, McNally T, Silverman M, Simons P, Stockton JD, Zambon MC. Rates of hospitalisation for influenza, respiratory syncytial virus and human metapneumovirus among infants and young children. *Vaccine*. 2006;24(1):102-108.

737. Care Quality Commission. University hospitals of leicester NHS trust leicester royal infirmary quality report. . 2014:09/07/2014.

738. World Medical Association. WMA declaration of helsinki - ethical principles for medical research involving human subjects. https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/. Updated 2013. Accessed Aug/ 21st, 2017.

739. National Institute for Health Research. Good clinical practice (GCP) reference guide. In: ICH Secretariat, ed. Geneva: ICH Secretariat; 2016:1-25. Accessed 21st Aug. 2017.

740. Centers for Disease Control and Prevention (CDC). Influenza like illness - CDC case definition. http://samaritanid.com/H1N1_symptoms.html. Updated 2009. Accessed August/ 21st, 2017.

741. Ogden TL.
The ISO and ACGIH standardized size fractions and their relation to human deposition data *The Annals of Occupational Hygiene*. 1988;32(Supplement 1):413-421.

742. Qiagen.
QIAsymphony DSP virus/pathogen kit instructions for use (handbook) . 2015.

743. Clark TW, Medina MJ, Batham S, Curran MD, Parmar S, Nicholson KG. C-reactive protein level and microbial aetiology in patients hospitalised with acute exacerbation of COPD. *Eur Respir J*. 2015;45(1):76-86.

744. Ellis JS, Curran MD. Simultaneous molecular detection and confirmation of influenza AH5, with internal control. *Methods Mol Biol*. 2011;665:161-181.

745. Emery SL, Erdman DD, Bowen MD, et al. Real-time reverse transcription-polymerase chain reaction assay for SARS-associated coronavirus. *Emerging Infectious Diseases*. 2004;10(2):311-316.

746. Kumar S, Stecher G, Tamura K. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol*. 2016;33(7):1870-1874.

747. Saitou N, Nei M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol Biol Evol*. 1987;4(4):406-425.

748. Dean AG, Sullivan KM, Soe MM. OpenEpi: Open source epidemiologic statistics for public health, version 3.01. www.openepi.com. Updated 06/04/2013. Accessed 01/15, 2016.

749. Monto AS, Bryan ER, Ohmit S. Rhinovirus infections in Tecumseh, Michigan: Frequency of illness and number of serotypes. *J Infect Dis*. 1987;156(1):43-49.

750. Hendley JO, Gwaltney JM,Jr. Mechanisms of transmission of rhinovirus infections. *Epidemiol Rev*. 1988;10:243-258.

751. Lessler J, Reich NG, Brookmeyer R, Perl TM, Nelson KE, Cummings DA. Incubation periods of acute respiratory viral infections: A systematic review. *Lancet Infect Dis*. 2009;9(5):291-300.

752. Minor TE, Dick EC, Peterson JA, Docherty DE. Failure of naturally acquired rhinovirus infections to produce temporal immunity to heterologous serotypes. *Infect Immun*. 1974;10(5):1192-1193.

753. Falsey AR. Respiratory syncytial virus infection in adults. *Semin Respir Crit Care Med*. 2007;28(2):171-181.

754. World Health Organization. *International health regulations (2005).* Second ed. Geneva: WHO Press; 2008.

755. Jartti T, Soderlund-Venermo M, Hedman K, Ruuskanen O, Makela MJ. New molecular virus detection methods and their clinical value in lower respiratory tract infections in children. *Paediatr Respir Rev*. 2013;14(1):38-45.

756. Stockton J, Ellis JS, Saville M, Clewley JP, Zambon MC. Multiplex PCR for typing and subtyping influenza and respiratory syncytial viruses. *J Clin Microbiol*. 1998;36(10):2990-2995.

757. Rakes GP, Arruda E, Ingram JM, et al. Rhinovirus and respiratory syncytial virus in wheezing children requiring emergency care. IgE and eosinophil analyses. *Am J Respir Crit Care Med*. 1999;159(3):785-790.

758. Rhedin S, Lindstrand A, Rotzen-Ostlund M, et al. Clinical utility of PCR for common viruses in acute respiratory illness. *Pediatrics*. 2014;133(3):e538-45.

759. Lee J. Mixed respiratory viral infections in children with adenoviral infections. *Infect Chemother*. 2016;48(4):347-349.

760. Abu-Diab A, Azzeh M, Ghneim R, et al. Comparison between pernasal flocked swabs and nasopharyngeal aspirates for detection of common respiratory viruses in samples from children. *J Clin Microbiol*. 2008;46(7):2414-2417.

761. Chan KH, Peiris JS, Lim W, Nicholls JM, Chiu SS. Comparison of nasopharyngeal flocked swabs and aspirates for rapid diagnosis of respiratory viruses in children. *J Clin Virol*. 2008;42(1):65-69.

762. Spyridaki IS, Christodoulou I, de Beer L, et al. Comparison of four nasal sampling methods for the detection of viral pathogens by RT-PCR-A GA(2)LEN project. *J Virol Methods*. 2009;156(1-2):102-106.

763. Andrewes C. Twenty years' work on the common cold. *Proc R Soc Med*. 1966;59(7):635-637.

764. Gwaltney JM,Jr, Hendley JO. Rhinovirus transmission: One if by air, two if by hand. *Am J Epidemiol*. 1978;107(5):357-361.

765. Douglas RG,Jr. Pathogenesis of rhinovirus common colds in human voluteers. *Ann Otol Rhinol Laryngol*. 1970;79(3):563-571.

766. Pancic F, Carpentier DC, Came PE. Role of infectious secretions in the transmission of rhinovirus. *J Clin Microbiol*. 1980;12(4):567-571.

767. Reed SE. An investigation of the possible transmission of rhinovirus colds through indirect contact. *J Hyg (Lond)*. 1975;75(2):249-258.

768. Parker J, Fowler N, Walmsley ML, et al. Analytical sensitivity comparison between singleplex real-time PCR and a multiplex PCR platform for detecting respiratory viruses. *PLoS One*. 2015;10(11):e0143164.

769. Kwok YL, Gralton J, McLaws ML. Face touching: A frequent habit that has implications for hand hygiene. *Am J Infect Control*. 2015;43(2):112-114.

770. Winther B, McCue K, Ashe K, Rubino J, Hendley JO. Rhinovirus contamination of surfaces in homes of adults with natural colds: Transfer of virus to fingertips during normal daily activities. *J Med Virol*. 2011;83(5):906-909.

771. Jennings LC, Dick EC. Transmission and control of rhinovirus colds. *Eur J Epidemiol*. 1987;3(4):327-335.

772. Winther B. Rhinovirus infections in the upper airway. *Proc Am Thorac Soc*. 2011;8(1):79-89.

773. Winther B, Brofeldt S, Christensen B, Mygind N. Light and scanning electron microscopy of nasal biopsy material from patients with naturally acquired common colds. *Acta Otolaryngol*. 1984;97(3-4):309-318.

774. Kennedy JL, Turner RB, Braciale T, Heymann PW, Borish L. Pathogenesis of rhinovirus infection. *Curr Opin Virol*. 2012;2(3):287-293.

775. Blair HT, Greenberg SB, Stevens PM, Bilunos PA, Couch RB. Effects of rhinovirus infection of pulmonary function of healthy human volunteers. *Am Rev Respir Dis*. 1976;114(1):95-102.

776. Cate TR, Couch RB, Johnson KM. Studies with rhinoviruses in volunteers: Production of illness, effect of naturallly acquired antibody, and demonstration of a protective effect not associated with serum antibody. *J Clin Invest*. 1964;43:56-67.

777. Rosenbaum MJ, De Berry P, Sullivan EJ, Pierce WE, Mueller RE, Peckinpaugh RO. Epidemiology of the common cold in military recruits with emphasis on infections by rhinovirus types 1A, 2, and two unclassified rhinoviruses. *Am J Epidemiol*. 1971;93(3):183-193.

778. Gwaltney JM. Clinical significance and pathogenesis of viral respiratory infections. *Am J Med*. 2002;112 Suppl 6A:13S-18S.

779. Camargo CN, Carraro E, Granato CF, Bellei N. Human rhinovirus infections in symptomatic and asymptomatic subjects. *Braz J Microbiol*. 2012;43(4):1641-1645.

780. Johnston SL, Sanderson G, Pattemore PK, et al. Use of polymerase chain reaction for diagnosis of picornavirus infection in subjects with and without respiratory symptoms. *J Clin Microbiol*. 1993;31(1):111-117.

781. Jackson DJ, Gangnon RE, Evans MD, et al. Wheezing rhinovirus illnesses in early life predict asthma development in high-risk children. *Am J Respir Crit Care Med*. 2008;178(7):667-672.

782. Jartti T, Korppi M. Rhinovirus-induced bronchiolitis and asthma development. *Pediatr Allergy Immunol*. 2011;22(4):350-355.

783. Kotaniemi-Syrjanen A, Vainionpaa R, Reijonen TM, Waris M, Korhonen K, Korppi M. Rhinovirus-induced wheezing in infancy--the first sign of childhood asthma? *J Allergy Clin Immunol*. 2003;111(1):66-71.

784. Cox DW, Bizzintino J, Ferrari G, et al. Human rhinovirus species C infection in young children with acute wheeze is associated with increased acute respiratory hospital admissions. *Am J Respir Crit Care Med*. 2013;188(11):1358-1364.

785. Palmenberg AC, Gern JE. Classification and evolution of human rhinoviruses. *Methods Mol Biol*. 2015;1221:1-10.

786. Tapparel C, Siegrist F, Petty TJ, Kaiser L. Picornavirus and enterovirus diversity with associated human diseases. *Infect Genet Evol*. 2013;14:282-293.

787. Tulve NS, Suggs JC, McCurdy T, Cohen Hubal EA, Moya J. Frequency of mouthing behavior in young children. *J Expo Anal Environ Epidemiol*. 2002;12(4):259-264.

788. Groot ME, Lekkerkerk MC, Steenbekkers LPA. *Mouthing behavior in young children: An observational study.* Wageningen, The Netherlands: Agricultural University Wageningen; 1998.

789. Irani DN. Aseptic meningitis and viral myelitis. *Neurol Clin*. 2008;26(3):635-55, vii-viii.

790. Racaniello VR. One hundred years of poliovirus pathogenesis. *Virology*. 2006;344(1):9-16.

791. Francis JR, Richmond P, Robins C, et al. An observational study of febrile seizures: The importance of viral infection and immunization. *BMC Pediatr*. 2016;16(1):202.

792. Enterovirus Foundation. About EV. http://www.enterovirusfoundation.org/aboutev_mid.shtml. Updated 2009. Accessed April/27, 2017.

793. Weber TP, Stilianakis NI. Inactivation of influenza A viruses in the environment and modes of transmission: A critical review. *J Infect*. 2008;57(5):361-373.

794. Pestre V, Morel B, Encrenaz N, et al. Transmission by super-spreading event of pandemic A/H1N1 2009 influenza during road and train travel. *Scand J Infect Dis*. 2012;44(3):225-227.

795. Pubic Health England. Surveillance of influenza and other respiratory viruses in the united kingdom: Winter 2013/ 2014. . 2014;PHE gateway number 2014110.

796. Public Health England. Surveillance of influenza and other respiratory viruses in the united kingdom: Winter 2014 to 2015. . 2015;PHE gateway number 2015046.

797. Matias G, Taylor R, Haguinet F, Schuck-Paim C, Lustig R, Shinde V. Estimates of hospitalization attributable to influenza and RSV in the US during 1997-2009, by age and risk status. *BMC Public Health*. 2017;17(1):271-017-4177-z.

798. Carrat F, Vergu E, Ferguson NM, et al. Time lines of infection and disease in human influenza: A review of volunteer challenge studies. *Am J Epidemiol*. 2008;167(7):775-785.

799. Talbot HK, Williams JV, Zhu Y, Poehling KA, Griffin MR, Edwards KM. Failure of routine diagnostic methods to detect influenza in hospitalized older adults. *Infect Control Hosp Epidemiol*. 2010;31(7):683-688.

800. Talbot HK, Falsey AR. The diagnosis of viral respiratory disease in older adults. *Clin Infect Dis*. 2010;50(5):747-751.

801. Clark TW, Ewings S, Medina MJ, et al. Viral load is strongly associated with length of stay in adults hospitalised with viral acute respiratory illness. *J Infect*. 2016;73(6):598-606.

802. Virlogeux V, Fang VJ, Wu JT, et al. Brief report: Incubation period duration and severity of clinical disease following severe acute respiratory syndrome coronavirus infection. *Epidemiology*. 2015;26(5):666-669.

803. Chaberny IE, Schnitzler P, Geiss HK, Wendt C. An outbreak of epidemic keratoconjunctivtis in a pediatric unit due to adenovirus type 8. *Infect Control Hosp Epidemiol*. 2003;24(7):514-519.

804. Montessori V, Scharf S, Holland S, Werker DH, Roberts FJ, Bryce E. Epidemic keratoconjunctivitis outbreak at a tertiary referral eye care clinic. *Am J Infect Control*. 1998;26(4):399-405.

805. Rice P. Viral transmission: Infection acquired by all other routes (respiratory, eye-nose-mouth, inoculation, and foeco-orally. In: Zuckerman AJ, Banatvala JE,

Schoub BD, Griffiths PD, and Mortimer P, eds. *Principles and practice of clinical virology.* 6th ed. Chichester: John Wiley & Sons, Ltd.; 2009:43.

806. Warren D, Nelson KE, Farrar JA, et al. A large outbreak of epidemic keratoconjunctivitis: Problems in controlling nosocomial spread. *J Infect Dis*. 1989;160(6):938-943.

807. Percivalle E, Sarasini A, Torsellini M, et al. A comparison of methods for detecting adenovirus type 8 keratoconjunctivitis during a nosocomial outbreak in a neonatal intensive care unit. *J Clin Virol*. 2003;28(3):257-264.

808. Rice P. Viral transmission: Infection acquired by all other routes (respiratory, eye-nose-mouth, inoculation, and foeco-orally). In: Zuckerman AJ, Banatvala JE, Schoub BD, Griffiths PD, Mortimer P, eds. *Principles and practice of clinical virology.* 6th ed. Chichester: John Wiley & Sons; 2009:43-64.

809. Putto A, Ruuskanen O, Meurman O. Fever in respiratory virus infections. *Am J Dis Child*. 1986;140(11):1159-1163.

810. Grayson SA, Griffiths PS, Perez MK, Piedimonte G. Detection of airborne respiratory syncytial virus in a pediatric acute care clinic. *Pediatr Pulmonol*. 2017;52(5):684-688.

811. Kulkarni H, Smith CM, Lee Ddo H, Hirst RA, Easton AJ, O'Callaghan C. Evidence of respiratory syncytial virus spread by aerosol. time to revisit infection control strategies? *Am J Respir Crit Care Med*. 2016;194(3):308-316.

812. Hall CB, Douglas RG,Jr, Schnabel KC, Geiman JM. Infectivity of respiratory syncytial virus by various routes of inoculation. *Infect Immun*. 1981;33(3):779-783.

813. Thorburn K, Kerr S, Taylor N, van Saene HK. RSV outbreak in a paediatric intensive care unit. *J Hosp Infect*. 2004;57(3):194-201.

814. Hall CB. The shedding and spreading of respiratory syncytial virus. *Pediatr Res*. 1977;11(3 Pt 2):236-239.

815. Garcia-Garcia ML, Calvo C, Rey C, et al. Human metapnuemovirus infections in hospitalized children and comparison with other respiratory viruses. 2005-2014 prospective study. *PLoS One*. 2017;12(3):e0173504.

816. Boivin G, De Serres G, Cote S, et al. Human metapneumovirus infections in hospitalized children. *Emerg Infect Dis*. 2003;9(6):634-640.

817. Freymouth F, Vabret A, Legrand L, et al. Presence of the new human metapneumovirus in french children with bronchiolitis. *Pediatr Infect Dis J*. 2003;22(1):92-94.

818. Nissen MD, Siebert DJ, Mackay IM, Sloots TP, Withers SJ. Evidence of human metapneumovirus in australian children. *Med J Aust*. 2002;176(4):188.

819. Peiris JS, Tang WH, Chan KH, et al. Children with respiratory disease associated with metapneumovirus in hong kong. *Emerg Infect Dis*. 2003;9(6):628-633.

820. Peret TC, Boivin G, Li Y, et al. Characterization of human metapneumoviruses isolated from patients in north america. *J Infect Dis*. 2002;185(11):1660-1663.

821. Paranhos-Baccala G, Komurian-Pradel F, Richard N, Vernet G, Lina B, Floret D. Mixed respiratory virus infections. *J Clin Virol*. 2008;43(4):407-410.

822. Shi T, McLean K, Campbell H, Nair H. Aetiological role of common respiratory viruses in acute lower respiratory infections in children under five years: A systematic review and meta-analysis. *J Glob Health*. 2015;5(1):010408.

823. Widmer K, Griffin MR, Zhu Y, Williams JV, Talbot HK. Respiratory syncytial virus- and human metapneumovirus-associated emergency department and hospital burden in adults. *Influenza Other Respir Viruses*. 2014;8(3):347-352.

824. Moisiuk SE, Robson D, Klass L, et al. Outbreak of parainfluenza virus type 3 in an intermediate care neonatal nursery. *Pediatr Infect Dis J*. 1998;17(1):49-53.

825. Zambon M, Bull T, Sadler CJ, Goldman JM, Ward KN. Molecular epidemiology of two consecutive outbreaks of parainfluenza 3 in a bone marrow transplant unit. *J Clin Microbiol*. 1998;36(8):2289-2293.

826. Lau SK, To WK, Tse PW, et al. Human parainfluenza virus 4 outbreak and the role of diagnostic tests. *J Clin Microbiol*. 2005;43(9):4515-4521.

827. Chughtai AA, Wang Q, Dung TC, Macintyre CR. The presence of fever in adults with influenza and other viral respiratory infections. *Epidemiol Infect*. 2017;145(1):148-155.

828. Lebet R, Fineman LD, Faustino EV, Curley MA. Asking for parents' permission to enroll their child into a clinical trial: Best practices. *Am J Crit Care*. 2013;22(4):351-356.

829. Sammons HM, Atkinson M, Choonara I, Stephenson T. What motivates british parents to consent for research? A questionnaire study. *BMC Pediatr*. 2007;7:12.

830. Fisher JA, Kalbaugh CA. Challenging assumptions about minority participation in US clinical research. *Am J Public Health*. 2011;101(12):2217-2222.

831. Leonard A, Hutchesson M, Patterson A, Chalmers K, Collins C. Recruitment and retention of young women into nutrition research studies: Practical considerations. *Trials*. 2014;15:23-6215-15-23.

832. Rooney LK, Bhopal R, Halani L, et al. Promoting recruitment of minority ethnic groups into research: Qualitative study exploring the views of south asian people with asthma. *J Public Health (Oxf)*. 2011;33(4):604-615.

833. Kumar S, Stecher G, Tamura K. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular biology and evolution*. 2015;Submitted.

834. National Library of Medicine (US). The NCBI handbook [internet]. chapter 18, the reference sequence (RefSeq) project. http://www.ncbi.nlm.nih.gov/books/NBK21091/. Updated Oct., 2002. Accessed June/30, 2016.

835. Tamura K, Nei M, Kumar S. Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proc Natl Acad Sci U S A*. 2004;101(30):11030-11035.