From DEPARTMENT OF WOMEN'S AND CHILDREN'S HEALTH
Karolinska Institutet, Stockholm, Sweden

VIRAL RESPIRATORY TRACT INFECTIONS IN CHILDREN

Johan Hamrin

Karolinska Institutet

Stockholm 2021

Published by Karolinska Institutet.
Printed by Universitetsservice US-AB, 2021

ISBN 978-91-8016-333-0
Cover illustration: Syncytia formation caused by eGFP-marked RSV infection of epithelial cells viewed in a UV-filtered microscope. Image taken by the author while conducting laboratory studies in Prof Anna-Lena Spetz research group at Stockholm University.

Viral Respiratory Tract Infections in Children

THESIS FOR DOCTORAL DEGREE (Ph.D.)

by

Johan Hamrin

The thesis will be defended in public at Rehabsalen, Norbacka S2:01 Eugeniavägen 39, Karolinska University Hospital
Friday 19th November 2021

Principal Supervisor:
Associate Professor Anna Nilsson
Karolinska Institutet
Department of Women's and Children's Health
Division of Pediatric Oncology and Surgery

Co-supervisor(s):
Associate Professor Margareta Eriksson
Karolinska Institutet
Department of Women's and Children's Health
Division of Clinical Pediatrics

Professor Eric Herlenius
Karolinska Institutet
Department of Women's and Children's Health
Division of Clinical Pediatrics

Professor Anna-Lena Spetz
Stockholm University
Department of Molecular Biosiences
Division of Infection and Immunobiology

Doctor Lars Björk
Karolinska Institutet
Department of Women's and Children's Health
Division of Pediatric Oncology and Surgery

Opponent:
Professor Johan Westin
University of Gothenburg
Institute of Biomedicine
Department of Infectious Diseases

Examination Board:
Associate Professor Maria Lundgren
Uppsala University
Department of Women's and Children's Health
Division of Perinatal, Neonatal and Pediatric Cardiology Research

Associate Professor Erik Eklund
Lund University
Department of Clinical Research, Section V
Division of Pediatrics

Senior Lecturer Marie Studahl
University of Gothenburg
Institute of Biomedicine
Department of Infectious Diseases

This is dedicated to my wife Thirza and our boys Harry, Hilmer and Casper.

POPULAR SCIENCE SUMMARY OF THE THESIS

As I am writing this thesis in October 2021, we are more than a year into the Covid-19 pandemic and the potential impact of respiratory viral infections on our society has never been more obvious. Our society has become acutely aware of the importance of understanding the transmission dynamics of a viral respiratory pathogen, which population is at risk for severe disease and which treatments are effective. The growing understanding of these issues have formed policies to inhibit transmission through measures such as various degrees of social distancing, self-isolation of populations at risk, individual measures (e.g. handwashing, use of disinfectants and face-masks), vaccination programs and adapted care of critically ill patients.

This thesis is focused on respiratory viral infections that have been circulating in society for a much longer time. In the case of influenza virus, it entered our awareness as the infamous 'Spanish Flu' pandemic in 1914. Genetic studies suggest that influenza and respiratory syncytial virus have been infecting humans for several hundred years. Apart from the older population and persons with mainly cardiovascular, respiratory, or immunological health issues, respiratory viral infections typically present as a simple cold. This is also mostly true for the vast majority of children. However, a small proportion of children, which becomes a large group in absolute numbers, require hospital admission during reoccurring viral epidemics. By improving our ability to predict epidemics we may improve healthcare resource allocation to a better extent. With better understanding of children at risk, more targeted prevention becomes possible. This includes both learning from the past as well as investigating new tools that can help us in the future.

The herein provided data strengthens the notion that epidemic predictions may be improved based on data regarding duration of immunity combined with birth rates. It also addresses the ongoing competition between respiratory viruses themselves as well as with our immune systems. Based on our findings, general climate conditions appear to be more important than meteorological changes. Identification of children at risk has already led to vaccination recommendations for influenza viruses and short-term, "borrowed" immunity through passive immunization. However, our data suggest that particularly children with decreased mobility due to diseases in their nervous system or muscles deserve more attention. Our results reveal that the risk to acquire and succumb to severe infection by respiratory syncytial virus is exceptionally high at birth with a steep decline in risk in the first few months. We also examine the potential of assessing the severity of infection with a urinary sample. Analysis from urinary samples collected from infants admitted to the hospital revealed an elevated marker among those with viral infections of their lower respiratory tract, such as pneumonia and bronchitis.

Hopefully, the knowledge gained from studies contained in this thesis takes us a step forward in preventing and treating the most common diseases in childhood.

ABSTRACT

The most common infection in humans is viral respiratory tract infections which predominantly present as the 'common cold'. In some circumstances however, respiratory viruses cause acute lower respiratory tract infections (RTIs) and due to the high absolute number of infection the contribution of these cases to total morbidity and mortality are substantial. For several reasons relating to physiology and immunology, children account for a great amount of morbidity caused by viral respiratory infections, namely influenza virus (IFV) and respiratory syncytial virus (RSV).

The aims of this thesis are to define burden and risk-factors associated with severe IFV and RSV infections among children. In papers **I**, **III**, **IV** and study **V** (in manuscript), we retrospectively investigated risk-factors, complications and epidemiological drivers associated with RSV and IFV based on hospitalized children 0-17 years old residing in the Northern Stockholm region. In total, these studies span over 21 years from 1st July 1998 until 30th June 2019. A total of 1,050 IFV and 5,253 RSV cases were included over that period.

Our results indicate that a steep rise in birth rates combined with viral interference caused by the pandemic H1N1 Influenza A virus (IFV-A pdm09) in 2009-10 were likely contributors to the disruption of the delayed biennial cyclicity of RSV epidemics. We derived that the cold winters coinciding with the altered epidemic pattern had a lesser role. Our data reveals a triennial pattern of IFV epidemics 2008 – 2019 and a rise in influenza type B (IFV-B) in the same period. It is not clear whether this is related to improved IFV-B diagnostics or a true change in IFV ecology.

Furthermore, we identified children with neuromuscular conditions to be at substantial risk of severe IFV infection, warranting improved vaccine coverage. Regarding RSV, our findings illustrate the importance of older siblings as a 'vector' for disease transmission to newborns, who are the most susceptible to severe disease during their first three months of life.

In paper **II**, the potential for the urinary PGE_2 metabolite (u-tPGEM) to identify children with severe viral infections, was investigated. We established baseline values for u-tPGEM in infancy and found higher levels among hospitalized children with lower RTIs and gastroenteritis, but not in infants with upper RTIs. Further studies are required to understand more about the immunological pathways involved in viral RTIs to predict disease progression in an individual and hopefully identify targets for future treatments.

Taken together, our findings improve the understanding of dynamics involved in seasonal epidemics which can help us to prepare what is to come in the wake of the Covid-19 pandemic. These results also indicate which group would benefit the most from prevention efforts. In the long-term, describing immunological pathways involved in severe viral respiratory infections can help us to finally advance from the current therapeutic plateau.

LIST OF SCIENTIFIC PAPERS

I. Samuel Rhedin, Johan Hamrin, Pontus Naucler, Rutger Bennet, Maria Rotzén-Östlund, Anna Färnert, Margareta Eriksson. Respiratory Viruses in Hospitalized Children with Influenza-Like Illness during the H1N1 2009 Pandemic in Sweden. PloS ONE 2012; 7: e51491.

II. Johan Hamrin, Monica Perez-Manzo, Helena Idborg, Per-Johan Jakobsson, Lars Björk, Margareta Eriksson, Anna Nilsson, Eric Herlenius. Urinary PGE_2 metabolite levels in hospitalized infants with infections compared to age-matched controls. Acta Paediatrica 2019; 108: 1879-1886.

III. Rutger Bennet, Johan Hamrin, Benita Zweygberg Wirgart, Maria Rotzén-Östlund, Åke Örtqvist, Margareta Eriksson. Influenza epidemiology among hospitalized children in Stockholm, Sweden 1998-2014. Vaccine 2016;

IV. Johan Hamrin, Rutger Bennet, Jonas Berner, Maria Rotzén-Östlund, Margareta Eriksson. Rates and risk factors of severe respiratory syncytial virus infection in 2008-2016 compared with 1986-1999. Acta Paediatrica 2020; 00:1-7.

V. Johan Hamrin, Rutger Bennet, Ida Hed Myrberg, Robert Dyrdak, Margareta Eriksson, Anna Nilsson. Exception makes the rule: Temporary disruption of the delayed biennial pattern of RSV epidemics in Stockholm. In manuscript.

SCIENTIFIC PAPERS NOT INCLUDED IN THE THESIS

Åke Örtqvist, Rutger Bennet, Johan Hamrin, Malin Ryd Rinder, Hans Lindblad, Joana Nederby Öhd, Margareta Eriksson. Long term effectiveness of adjuvanted influenza A(H1N1)pdm09 vaccine in children. Vaccine 2015; 33: 2258-2561

Helena Idborg, Sven-Christian Pawelzik, Monica Perez-Manso, Lars Björk, Johan Hamrin, Eric Herlenius, Per-Johan Jakobsson. Evaluation of urinary prostaglandin E2 metabolite as a biomarker in infants with fever due to viral infection. Prostaglandins, Leukotrienes and Essential Fatty Acids 2014; 91: 269–275

CONTENTS

LIST OF ABBREVIATIONS

A549	Human adenocarcinoma alveolar basal epithelial cell line
ACE2	Angiotensin Converting Enzyme 2
AdV	Adenovirus
ALRI	Acute Lower Respiratory Tract Infection
AOM	Acute Otitis Media
APP	Acute Phase Proteins
BoV	Bocavirus
CI	Confidence Interval
CoV	Coronavirus
CoV-SARS-2	Coronavirus responsible for Covid-19 pandemic
COX-1	Cyclooxygenase 1
COX-2	Cyclooxygenase 2
CPAP	Continuous Positive Airway Pressure
CPE	Cytopathologic Effects
CRP	C-Reactive Protein
CSF	Cerebrospinal Fluid
DNA	Desoxyribonucleic Acid
ds	double stranded
eGFP	enhanced Green Fluorescent Protein
EIA	Enzyme Immunoassay
EV	Enterovirus
GE	Gastroenteritis
GFR	Glomerular Filtration Rate
H or HA	Hemagglutinin
HIV	Human Immunodeficiency Virus
HN	Hemagglutinin-Neuraminidase
HSCT	Hematopoietic Stem Cell Treatment
HSV	Herpes Simplex Virus
ICAM-1	Intracellular Adhesion Molecule 1
IF	Immunofluorescence

IFITM	Interferon-Inducible Transmembrane Protein
IFN	Interferon
IFV	Influenza virus
IFV-A	Influenza virus type A
IFV-A	Pandemic influenza of 2009 (H1N1 Influenza A pdm09)
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IIVs	Inactivated Influenza Vaccines
IL-10	Interleukin 10
IQR	Interquartile Range
LAIVs	Live Attenuated Influenza Vaccines
LC-MS/MS	Liquid Chromatography Tandem Mass Spectrometry
LRTI	Lower Respiratory Tract Infection
M2	Matrix protein 2
MERS	Middle East Respiratory Syndrome
MPV	Metapneumovirus
N or NA	Neuraminidase
NGS	Next Generation Sequencing
NSAID	Non-Steroidal Anti-Inflammatory Drug
OR	Odds Ratio
PCR	Polymerase Chain Reaction
PCT	Procalcitonin
PGE_2	Prostaglandin E_2
PICU	Pediatric Intensive Care Unit
PIV	Parainfluenza virus
PRR	Pattern Recognition Receptors
RIV	Recombinant Influenza Vaccines
RNA	Ribonucleic Acid
RSV	Respiratory Syncytial Virus
RT-PCR	Real-Time PCR

RTI	Respiratory Tract Infection
RV	Rhinovirus
SARS	Severe Acute Respiratory Syndrome
SIRS model	Susceptible – Infected – Recovered – Susceptible model
SOT	Solid Organ Transplant
ss	single stranded
u-tPGEM	Urinary tetranor Prostaglandin E_2 metabolite
URTI	Upper Respiratory Tract Infection
VP	Virion protein

1 INTRODUCTION

Based on a global data collection from a period spanning over 26 years, acute lower respiratory infections (ALRIs) were estimated to have caused 652 572 deaths in children under 5 years in the year of 2016. Approximately 75% of these deaths caused by ALRIs occur during the first year of life and more than half are attributed to *Streptococcus pneumoniae* followed by *Haemophilus influenzae* type b, to which only 7% are attributed [1]. With improved vaccine coverage to the aforementioned bacteria and access to antibiotics, recent advances in the prevention of deaths are set to continue. Notably, viral infections, namely respiratory syncytial virus (RSV) and influenza virus (IFV), are the third and fourth most common causes of death due to ALRIs. Estimates from a meta-analysis based on studies spanning over 20 years in 132 countries concluded that RSV-infections globally led to 3.2 million annual hospital admissions in children <5 years of age, 50% of these in children younger than 6 months [2]. The corresponding estimate of hospital admissions in children <5 years due to influenza was 1 million cases for 2008 [3]. In addition to RSV and IFV, the following respiratory viruses have shown strong evidence to cause ALRIs in children <5 years: Parainfluenzavirus (PIV), Metapneumovirus (MPV) and to a lesser extent Rhinovirus (RV). Findings of Adenovirus (AdV), Bocavirus (BoV) and seasonal Coronaviruses (CoV) have not differed among children with ALRIs compared to healthy controls [4,5]. CoV-SARS-2 is the virus responsible for the current Covid-19 pandemic which has been generally much milder in children, but with notable exceptions [6,7].

Deaths in children due to ALRIs in general, and ALRIs caused by viruses in particular, are rare events in developed countries. However, high hospital admission rates during reoccurring winter epidemics in temperate climates is strenuous for health care systems as hospital beds are filled with children requiring treatment for ALRIs. In the case of RSV epidemics in Stockholm 1987 - 1998, approximately 1% of all infants in the hospital's catchment were admitted each winter with 10% of these requiring treatment at the pediatric intensive care unit (PICU) [8]. As a result, the hospitals capacity to deliver elective care for other children is repeatedly diminished during certain weeks of the year.

The aim of this thesis is to advance the understanding of viral infections in children in respect to factors that control the timing and magnitude of viral epidemics and which children are at risk for severe infection and to add a potential diagnostic tool. These research questions are addressed in several retrospective studies as well as a prospective, clinical study.

Our results have implications for predicting seasonal viral epidemics, prevention measures and identifies a potential role for prostaglandin E_2 as a marker for severe viral infection.

2 LITERATURE REVIEW

2.1 DETECTION OF RESPIRATORY VIRUSES

Respiratory viruses are typically diagnosed by specimens collected from the upper respiratory tract, predominantly from the nose. Mucus may be collected with a swab or by a nasopharyngeal aspirate. Tracheal aspirates may be collected in intubated patients.

Some respiratory viruses, such as AdV and Enteroviruses (EVs), may also affect the gastrointestinal tract in which case the respective virus can be identified in fecal samples. In addition, certain respiratory viruses such as EVs can cause infections in the central nervous system, which can be diagnosed through virus detection in the cerebrospinal fluid.

The main methods of viral detection currently in use in clinical diagnostics are viral cultures, antigen detection and amplification of viral genetic codes. Unless otherwise specified the following chapters 2.1.1 – 2.1.3 are based on the detailed description of viral detection methods in Fields Virology, 5th edition [9].

2.1.1 Virus isolation in cell cultures

Viral cultures stand apart from other diagnostic methods for several reasons: a) viable virus particles are a prerequisite, b) the particles must be able to infect the cell-type used in the cell culture and replicate in them, c) obtained virus isolates through this amplification process provides material for further characterization d) also unsuspected and/or unknown viruses may be detected.

In most cases, cultures of immortalized, surface-adherent cells that can be passaged without limit are used for diagnostic purposes such as Hep-2, HeLa, A549 and Madin-Darby canine kidney (MDCK) cells (**Figure 1**). Primary human host cells for the specific respiratory virus, usually respiratory epithelial cells, are sometimes used for research purposes to mimic pathologic processes within humans. The most advanced of these primary epithelial cell models are grown in an air-liquid interface, where the cell layers establish cilia at the apical surface as they do in human airways [10].

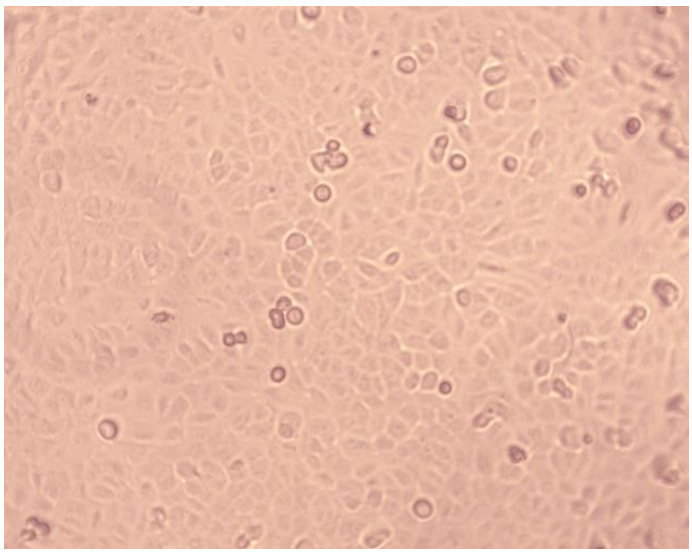

Figure 1: A549 adenocarcinomic human aleveolar basal epithelial cell culture observed in light microscope. (author's image)

Using light microscopy, virus-induced changes in the cell culture are called cytopathologic effects (CPEs). CPEs are an indirect method for viral detection but can be quite specific for certain viruses. One characteristic CPE of RSV is the formation of syncytia resulting from fusion of adjacent epithelial cells and has also given rise to the virus name (**Figure 2**). In a research setting, an additional transcription unit may be inserted into the viral genome coding for a marker such as enhanced green fluorescent protein (eGFP), allowing for visualization of virus-infected cells (**Figure 2**) [11].

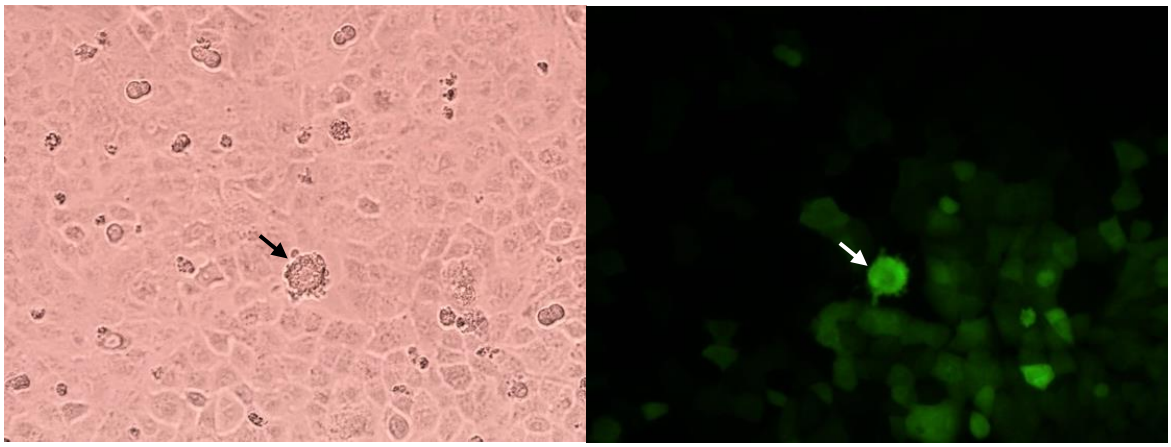

Figure 2: Syncytia induced by RSV infection in A549 cell lines as seen under light microscope (left). The right image depicts the same cell surface seen through an eGFP filtered microscope revealing RSV-infected cells. (Author's images)

As CPEs rarely are virus-specific and dependent on the cell type on which it is cultured, additional methods are required for characterization and identification of the virus. Viral particles are visible with electron microscopy allowing for further characterization. This method is particularly useful when the origin of the virus in the sample is unknown. The most common virus-specific detection methods are either based on antigen detection or amplification of viral gene codes. These methods are useful when there is a strong suspicion of a specific virus. Viral cultures are cumbersome and time-consuming as it usually takes days to several weeks to ascertain a negative finding. In clinical practice, they have been replaced by more sensitive, faster, high-throughput methods [12,13].

Aside from research, viral cultures still have a role in identifying new viruses or significant genomic changes in viruses rendering the specific methods less sensitive or even false-negative in the context of infectious disease surveillance.

2.1.2 Antigen Detection

The possibility to enhance virus-infected cells in a microscope through labelling with fluorescent antibodies, called immunofluorescence (IF), has been present since the 1950s [14]. This method requires intact cellular structures in the collected specimen. Cell-associated viral antigens are bound by specific antibodies that can be coupled with a fluorescent label (direct IF). A variant of IF, called indirect IF, couples anti-immunoglobulin antibodies with the fluorescent label, which in turn identifies bound virus-specific antigens. The latter method is usually more sensitive. Using ultraviolet light at the specific wavelength for the fluorescent label, virus-infected cells become visible under the microscope. Usually, normal cellular structures are visualized with a different color label using the same method to generate a background. IF has been used in combination with viral culture in routine clinical diagnostics at the Karolinska University Hospital in Stockholm from 1993 – 2007 [12,13].

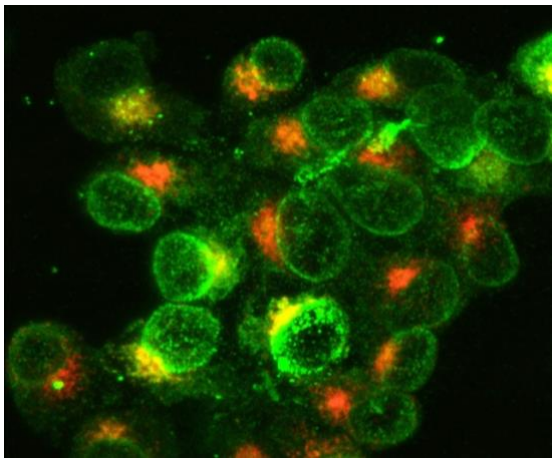

Figure 3: Image of cell surface proteins enhanced by immunofluorescence viewed in a UV-filtered microscope. Image including license for publication purchased from iStock.

Another viral antigen detection method is enzyme immunoassay (EIA). In contrast to IF, EIA does not require an intact cellular structure which improves its applicability. Viral antigens within a specimen are bound using a 'capture' antibody residing on a capture surface. In a second step, captured viral antigens are detected with another, enzyme-labeled antibody. Similar to the aforementioned direct and indirect IF methods, enzymes may be labeled to viral antigen-antibodies directly or indirectly with an anti-immunoglobulin antibody. The enzyme produces a color change or light emission with the addition of an enzyme substrate to the sample. Membrane immunoassays function as EIAs while directly capturing antigens or antigen-antibodies to membranes. This method is applied in commercial kits, which can be used as bedside tests providing results within 15 minutes though with a relative loss in sensitivity and specificity [15,16]. Commercial rapid antigen tests have been in use at Astrid Lindgren Children's Hospital, Karolinska University Hospital during RSV epidemics from 2007 – 2019. Their main purpose has been to facilitate cohort care of infected children to minimize nosocomial RSV infection among other hospitalized children.

2.1.3 Genomic code detection

2.1.3.1 Polymerase chain reaction

The most established viral detection method is polymerase chain reaction (PCR), based on amplification of specific codes of nucleic acids within the viral genome. A prerequisite for this method is the availability of sufficiently specific and stable codes within the viral genome to reliably identify a virus. Inherent to the method is that it does not require an intact virus or infected cell, which both improves sensitivity and facilitates handling of clinical samples.

In a first step, nucleic acids are extracted through lysis of cells and virions. Extracted nucleic acids are subsequently analyzed in repeated temperature-regulated cycles comprising of denaturation, primer annealing and polymerization of the DNA-strand into double stranded DNA (dsDNA). This process requires a thermostable polymerase enzyme, premanufactured oligonucleotide primers targeting the intended region within the viral genome and sufficient nucleotides. Apart from DNA-viruses AdV and BoV, most viral agents in the respiratory tract are RNA-viruses. Identification of RNA-viruses therefore require an additional step using an enzyme called reverse transcriptase, which converts RNA sequences into its DNA counterpart. The PCR-product, called amplicon, can be detected and indirectly quantified as its produced in a process called real-time PCR (RT-PCR). One common RT-PCR method utilizes a DNA binding dye called SYBR Green, which becomes fluorescent when bound to dsDNA. The more viral particles a sample contains, the fewer cycles are needed to detect an amplicon. In general, amplicons detected around approximately 40 cycles may be unspecific which is why it is often used as a cut-off value. A further development of PCR is multiplex PCR where several primers are added to the reaction, allowing for detection of several viruses within the same analysis. The high sensitivity and specificity, combined with short analysis duration has made PCR the gold standard for detection of most clinically relevant viruses,

respiratory viruses in particular [13]. The 'Achilles' heel' of PCR-diagnostics is mutations in the targeted codon of the genome, which can result in significantly decreased sensitivity if not false-negative results. This was the case for the H1N1 influenza pdm09 strain, whose genome differed in four base-pairs within the codon targeted by existing PCR methods for Influenza A detection [17,18]. Albeit an intracellular bacterium, not a virus, *Chlamydia trachomatis* is also diagnosed with PCR technique since 1990s. In the period 2005 – 2006 a sudden drop in diagnosed chlamydia cases was caused by a new chlamydia strain carrying a deletion in part of the genomic codon targeted by the PCR method in place [19]. These examples stress the need for the aforementioned virus detection methods for surveillance purposes.

2.1.3.2 Whole genome sequencing

Whole genome sequencing provides the entire genetic code of a species. The technique has been available since the 1970s with significant advances in the 1990s, followed by high throughput "next generation sequencing" (NGS) methods in the 2000s [20]. In the case of respiratory viral infections, sequencing has thus far not found a role in the routine clinical setting. However, the method does provide the possibility to understand phylogenetics, antiviral resistance patterns as well as improving vaccine development [21–23].

2.1.4 Serology

Serologic analyses are an indirect method to detect an ongoing, but more often a past infection with a virus through measurement of specific immune responses. Immunoglobulin M (IgM) antibodies in a primary infection can be detected within 5-10 days after exposure to a virus. These are typically of lower antigen affinity and therefore less specific. Starting around three weeks from initial exposure Immunoglobulin G (IgG) antibodies, with higher antigen affinity become detectable. Serum levels of IgGs tend to peak 3-7 weeks after disease onset. Upon rechallenge with the same virus, humoral responses are mounted within 1-3 days constituted by predominantly IgGs with high affinity. Because repeated infections with respiratory viruses are very common, serologic tests utilize Ig types, levels and their affinity to distinguish between current and past infections. In many situations, an additional sample collected approximately 8 weeks after disease onset, called convalescent serum, is required to follow antibody kinetics [24–26]. Antibodies are typically measured using binding assays such as the previously described EIA method. Neutralizing assays measure the ability of antibodies to inhibit viral infection in a cell culture. The highest antibody dilution capable of inhibiting viral infection is determined as the neutralizing titer. The neutralizing titer relates to the affinity of the antibody as well as the importance of the specific epitope for viral infectivity [27]. Lower sensitivity of serologic assays and the frequent need for convalescent serum diminish the utility of serology in respiratory viral infections in a clinical setting. However, serology has an important role in epidemiologic studies on past exposure to these viruses [28–30].

2.1.5 Indirect biochemical markers of viral infections

Biochemical markers, such as C-reactive protein (CRP) and procalcitonin (PCT) have proven their use in identifying and monitoring bacterial infections, unfortunately these markers have limited use in severe viral infections [31–33]. Other acute phase proteins (APPs) have been evaluated in infants as markers for severe disease but with insufficient sensitivity [34]. The myxoma resistance protein 1 has shown more promising results for discriminating children with viral infections from those with bacterial or no infection, but the analysis is not available for routine diagnostics [35].

2.2 CLINICAL PRESENTATIONS OF VIRAL RESPIRATORY TRACT INFECTIONS

The clinical picture of viral infections of the respiratory tract is much more related to which specific part of the respiratory tract is infected than to the specific viral agent. Hence, there is a substantial overlap between clinical presentations of viral respiratory tract infections (RTIs) [36]. In addition, a virus may infect various parts of the respiratory tract at the same time leaving a mixed clinical picture. Another characteristic of viral RTIs is the lack of specific treatments and therefore treatments are focused on symptom relief. This chapter briefly presents the clinical picture of RTIs in children based on the anatomical site of the infection and principles of treatment. Clinical manifestations specific to each virus, targeted treatments, prophylaxis and vaccinations will be discussed in ensuing chapters dedicated to each virus. Unless otherwise specified, the following general descriptions of upper and lower respiratory tract infections are based on Principles and Practice of Infectious Diseases, 8th Edition and Nelson Textbook of Pediatrics, 18th Edition [37,38].

2.2.1 Upper Respiratory Tract Infections

The upper respiratory tract is defined as upper airways from nose and mouth stretching to and including the larynx (see **Figure 4**).

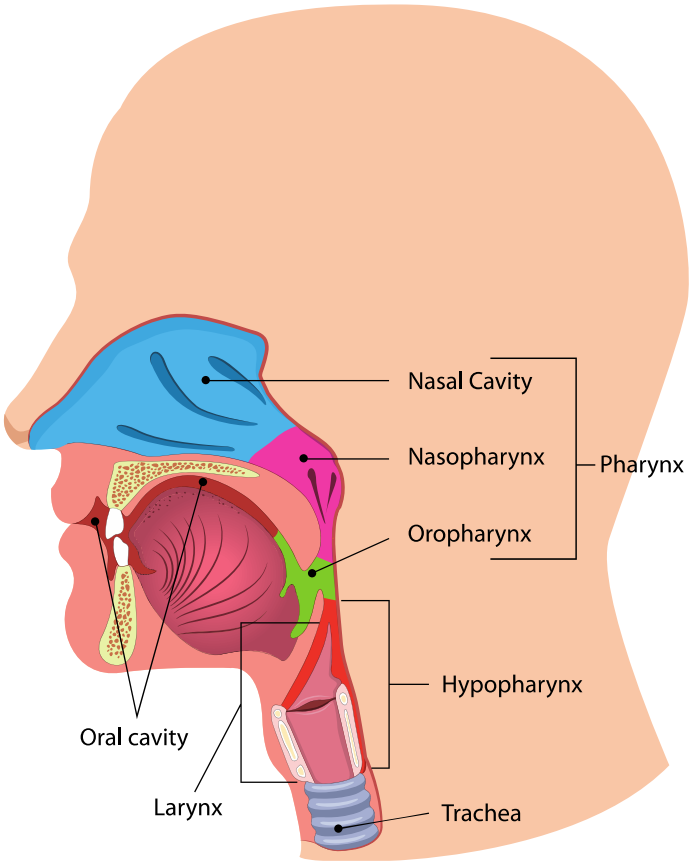

Figure 4. Anatomy of the upper respiratory tract. Image including license for publication purchased from iStock.

2.2.1.1 Rhinitis

Infectious rhinitis or coryza is a viral infection and the main presentation of what is referred to as the 'common cold'. The common cold may be the most common disease in humans, occurring 5 to 7 times a year in children and 2 to 3 times a year in adults. The clinical picture is characterized by rhinorrhea and nasal obstruction while often associated with a sore throat, coughing, malaise, headache and sometimes fever. Symptoms typically resolve within one week from onset. Symptomatic treatments are nasal decongestants, acetaminophen and non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen.

2.2.1.2 Pharyngitis

Acute pharyngitis typically presents with a sore throat, fever and pharyngeal inflammation. On physical examination, it manifests itself as pharyngeal erythema and edema with or without exudates, vesicles and ulcerations. More than 50% of the cases are attributed to bacterial infections and even among viral causes, non-respiratory viruses such as Epstein-Barr virus, herpes simplex virus, human cytomegalovirus and human immunodeficiency virus type 1 (HSV) may cause pharyngitis. Aside from HSV and HIV, treatment is symptomatic with acetaminophen and NSAIDs to reduce pain from sore throat, fever and malaise. Dehydration and insufficient nutrition due to the sore throat and loss of appetite occasionally requires treatment with intragastric or intravenous fluids in infants and small children. Symptoms may be present for up to two weeks.

2.2.1.3 Otitis Media

The middle ear is connected to the nasopharynx via the eustachian tube as well as posteriorly to the mastoid air cells. Acute otitis media (AOM) arises with inflammation to the mucosa in the middle ear space with fluid causing fever, significant pain and impaired hearing. Etiology can be viral, bacterial or both. AOM has an age-dependent incidence peaking in the first three years of life, when a one of three children will have succumbed to three or more AOM episodes. A second incidence peak has been noted at the time of school entry. The predominance of viral etiologies supports symptomatic treatment with nasal decongestants and pain relief in children older than two years of age with a mild clinical presentation. Clinical symptoms typically subside within less than a week, but middle ear fluid may remain weeks up to months after the infection leading to varying degrees of hearing loss.

2.2.1.4 Laryngotracheitis

Acute laryngotracheitis is often referred to as croup or false croup. The term false croup describes viral acute laryngotracheitis and is meant to differentiate from to the bacterial disease diphtheria, otherwise known as membranous or true croup. The clinical picture is defined by subglottic inflammation resulting in inspiratory stridor and a characteristic 'barking' cough. Symptoms typically occur and worsen at night. Incidence peaks at two years of age and is rarely seen in children older than six years. Studies have consistently found high associations with parainfluenza viruses, which have been found in approximately 80% of

positive findings. A single dose of cortisone has been shown to effectively reduce the need for hospitalization. Inhalations with epinephrine may also give temporary relief. In clinical practice, the effect of inhalations must be weighed against the detrimental effect of a frightened child whose increased circulation and blood-pressure will aggravate the mucosal swelling. In milder cases it is recommended to take the child outside to breathe cold air, which is thought to lead to mucosal vasoconstriction thereby reducing the mucosal swelling. The most severe symptoms seldom persist for more than two days and the infection is usually resolved within less than four days.

2.2.1.5 Sinusitis

Acute sinusitis is a bacterial infection which may arise as a complication to a viral URTI. This complication is estimated to occur in 6-13% of children with viral URTIs.

2.2.2 Lower Respiratory Tract Infections

The lower respiratory tract encompassed the airways below the larynx including the alveoli where gas-exchange takes place (**Figure 5**).

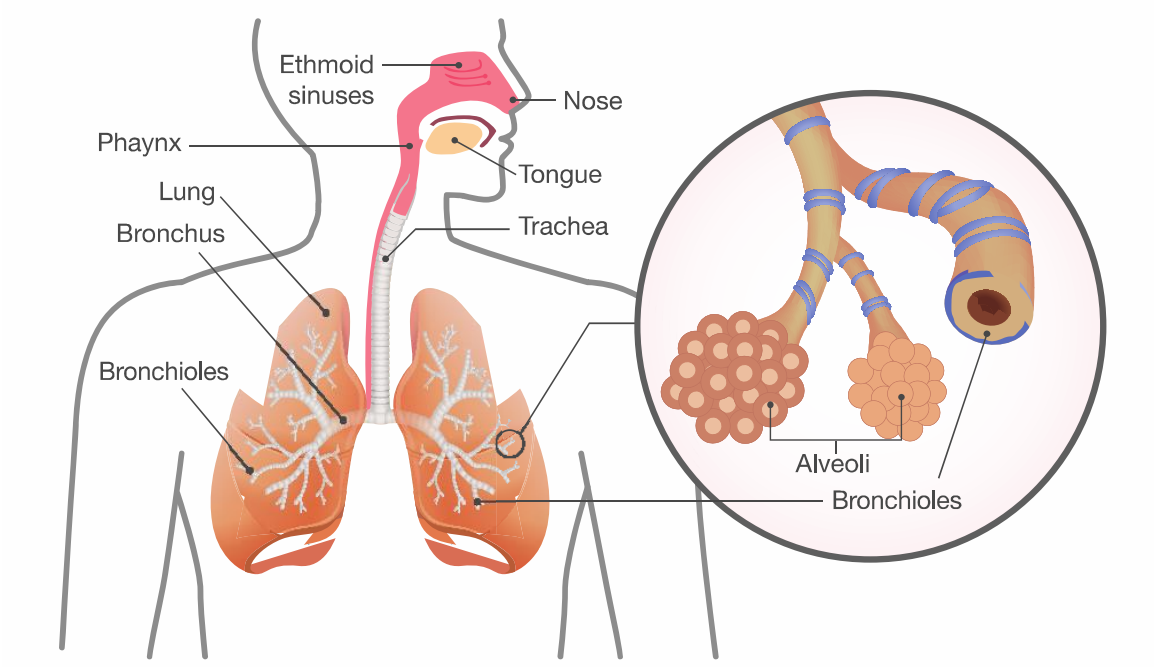

Figure 5. Anatomy of the lower respiratory tract. Image including license for publication purchased from iStock.

2.2.2.1 Tracheitis

Acute tracheitis is a bacterial infection that typically presents in younger children as a complication to a preceding viral URTI. It is rarely seen in school-aged children.

2.2.2.2 Bronchitis and Bronchiolitis

Acute bronchitis and bronchiolitis arise from infection and subsequent inflammation of the bronchi and bronchioles respectively (see **Figure 5**). Differentiating these two entities is challenging and some authors simply distinguish by the age at which a child first presents with obstructive symptoms: bronchiolitis < 2 years of age, bronchitis > 2 years of age, or subsequent episodes in children < 2 years. Viral infections are estimated to cause approximately 90% of all cases of acute bronchitis and often present initially as an acute URTI. Inflammation in the lower respiratory airways leads to a mucosal edema, production of mucus with subsequent plugging and spasms to the peribronchial muscles resulting in airway obstruction. The airway obstruction manifests itself as wheezing due to turbulence in the expiratory airflow, increased respiratory distress and a productive cough. Typically, the production of mucous is more pronounced with bronchiolitis. In addition to fever and the previously mentioned concomitant symptoms stemming from the upper respiratory tract, dehydration may also become pronounced in more severe cases. Treatment of bronchitis focuses on restoring patency of airways by inhalations with bronchodilators, primarily salbutamol and sometimes with the addition ipatropium bromide. Corticosteroids have a better effect when an asthmatic component is suspected in which case it is inhaled, given orally or rarely intravenously. Neither bronchodilators nor corticosteroids shorten disease duration or halt disease progression in bronchiolitis [39,40]. Nevertheless, inhalations with saline and/or epinephrine are often attempted in bronchiolitis-cases as they are perceived to achieve short-term clinical improvements [41]. Children requiring hospital admission often require feeding via nasogastric tubes or intravenous fluids due to dehydration. Insufficient oxygenation may require administration of oxygen through a cannula. In cases with severe obstruction which often coincide with partial atelectasis to the lung, oxygenated and nebulized air may be given through a high-flow cannula. Pediatric intensive care unit (PICUs) may provide more advanced respiratory support with continuous positive airway pressure (CPAP) or mechanical ventilation for children progressing to respiratory insufficiency.

2.2.2.3 Pneumonia

Pneumonia results from the inflammatory response to infection in the alveoli with various degrees of interstitial involvement. Typically, children present with tachypnoea, signs of respiratory distress and fever. Abdominal pain is common in lower lobe pneumonia and may also occur due to gastric distension caused by swallowed air. As with bronchitis and bronchiolitis, it is challenging to clinically delineate between pneumonia and other LRTIs in children. To standardize outcomes of vaccine studies, the World Health Organization utilizes a standardized chest radiograph description of ‘alveolar consolidation’. Prior to vaccination for *Hemophilus influenzae* type B and *Streptococcus pneumoniae,* it was estimated that bacteria accounted for approximately half of pneumonias in children [42]. In Sweden, vaccination against *H. influenzae* type B and *S. pneumoniae* were introduced in 1993 and 2009 respectively [43]. In follow-up studies in different countries after introduction of these vaccination programs, 73-87% of pneumonias have been attributed to viruses [5,44,45]. An

important challenge when comparing the effect of vaccinations against the most common bacteria is the substantial improvement and increased use of viral diagnostics in the same time frame [46]. Even when viral etiology is suspected, antibiotics may be administered in severe cases to prevent or to treat a possible bacterial superinfection. Unless there is a mixed clinical picture with bronchitis and/or bronchiolitis, inhalations are not effective. Mostly, hospital admissions focus on delivering supportive care to keep the child nourished and hydrated and to compensate for respiratory insufficiency.

2.2.3 Non-respiratory symptoms associated with viral RTIs

2.2.3.1 Seizures

Febrile seizures are a common and benign seizure disorder in childhood that occurs in children 9 months to 5 years of age, typically in association with a respiratory tract infection. The incidence is 3-4% at the age of peak onset, which is 14-18 months of age [37]. In a Hong Kong based study based on 923 admissions due to febrile seizures, 80% had a respiratory tract infection. Of the four analyzed respiratory viruses, IFV was the most common finding with 18%, followed by AdV 7%, PIV 6% and RSV in only 3% [47,48].

2.2.3.2 Encephalitis

Children with encephalitis may present with altered consciousness, lethargy, irritability persisting for at least 24 hours in combination with at least two of the following: fever, seizures and/or focal neurologic findings, pleocytosis in the cerebrospinal fluid (CSF), electroencephalogram or neuroimaging results concordant with encephalitis [49]. An Australian, multicenter study on children <14 years of age identified 287 cases of encephalitis in a two-year period. Among these, 10% were attributed to EVs and Parechoviruses respectively, 6% to IFV and 1% to RSV and AdV respectively [50].

2.2.3.3 Gastrointestinal symptoms

Vomiting and diarrhea and nausea are associated with viral RTIs in 30-50% among hospitalized children even for viruses that primarily do not replicate in the gastrointestinal tract in humans [51,52].

2.2.3.4 Myositis

Myositis is a rare complication associated with IFV and mainly occurs among school-aged children (boys vs girls: 2:1). Typically, myalgia in the calf muscles manifest approximately 3 days after disease onset. The course of disease is mostly benign with symptoms and elevations of muscular enzymes subsiding within days. In up to 10% of cases however, myositis leads to rhabdomyolysis resulting in kidney failure [53].

2.2.3.5 Myocarditis

Myocarditis is a rare complication of predominantly associated EVs with disease severity spanning from increases release of myocardial proteins to the blood stream without symptoms to cardiac failure necessitating PICU treatment [54–56].

2.2.4 Risk factors for severe viral RTIs

2.2.4.1 Infancy, premature birth and low birth weight

Airway size is an important factor when assessing children, especially infants with respiratory infections. Based on Hagen-Poiseuille's equation for laminar flow in a tube, the resistance is inversely related to the fourth power of the radius. Hence, the smaller the airways are to begin with, the more important partial obstructions become. Additionally, the high metabolic rate and smaller energy reserves in infants contribute to fatigue at an earlier stage than adults. Furthermore, the immune system of infants is adapting from life *in utero* which renders them more susceptible to viral infections [57,58]. All these factors combine in premature and low-birthweight infants, which are consistently identified as at risk for severe LRTIs [44,59–62]. The risk of acquiring late-onset-septicemia in the first months of life may also contribute to the admission of infants presenting with fever for observation of suspect septicemia. In many cases, the cause of the fever is a viral URTI, not in itself requiring hospitalization [63,64]. The use of procalcitonin as an additional marker for late onset sepsis has reduced this issue to some extent [65].

2.2.4.2 Male sex

Male sex has repeatedly been shown to be a risk factor for lower respiratory tract infections in children, with the strongest discrepancy in infancy [59,66–70]. Several of these meta-analyses report an OR of 1.25 for male infants regarding LRTIs. It has been suggested that the balance between immunotolerance and immunoreactivity is tilted differently between the sexes with females having more immunoreactivity resulting in better defense mechanisms for infectious diseases at the expense of more autoimmune diseases [71].

2.2.4.3 Siblings, daycare attendance and crowding

Since young age is an important risk-factor, crowded households and siblings impact how early and how much an infant will be exposed to viral respiratory tract infections. Daycare attendance has the same effect after infancy [59,72–75].

2.2.4.4 Socioeconomic factors

Perhaps also a surrogate marker for increased risk of crowding and environmental exposures, low socioeconomic status has also been associated with an increased risk of hospitalization [76,77].

2.2.4.5 Breast feeding

Breast feeding has a protective effect due to transmission of Immunoglobulin A (IgA), which is active on mucosal surfaces and perhaps due to altered colonization with microbiota [78]. During the first 6 months of life, breast feeding has a protective effect against multiple infections, including viral respiratory tract infections [59,72]. The protective effect also correlates with the amount of breastfeeding in relation to other nutritional sources [79].

2.2.4.6 Environmental exposures

Maternal use of nicotine from cigarettes, nicotine replacements and snuff have been shown to affect lung development *in utero* and increase the risk for respiratory infections and asthma in the child [80]. Independent from maternal smoking during pregnancy, household smoking has consistently been shown to increase the risk of LRTIs in children [80,81]. Ambient air pollution, particularly in urban areas, have also been consistently associated with increased risk of LRTIs in children [82,83].

2.2.4.7 Neurologic disorders

Children with neuromuscular disorders are at risk for severe ALRIs and other complications associated to viral infections. From a respiratory perspective, reduced muscular airway tonus, impaired movement coordination and muscular strength lead to a diminished ability to mobilize mucous. This results in mucous stagnation followed by airway obstruction which impedes viral clearance, aggravates the inflammatory response and increases the susceptibility to bacterial co-infections [84–86]. From a neurologic perspective, viral infections may in themselves cause seizures or exacerbate a pre-existing epileptic disorder [87,88].

2.2.4.8 Cardiopulmonary disorders

Given the strain on the cardiopulmonary organs a LRTI poses, it is unsurprising that chronic lung diseases and cardiac malformations are consistently identified as risks for severe LRTIs in children up to 2 years of age [59,62,89]. Asthma is often associated with an increased risk for hospitalization with an LRTI, however there are conflicting reports regarding subsequent risk for PICU admission and the duration of illness [61,62,90]. These variations may be explained by differences in criteria for hospitalization in different institutions and varying access to health care as well as compliance to asthma treatment in the studied populations.

2.2.4.9 Oncologic and hematologic disorders

Children with oncologic or hematologic disease, including immunodeficiencies and children under immunosuppressive treatment are often combined as a group in analysis for severe viral LRTIs, in particular with respect to influenza [62]. Hematopoietic stem cell treatment (HSCT) and solid organ transplant treatment (SOT) are associated with higher morbidity and mortality [91–93]. In analogy to infants < 3 months of age, immunocompromised children, particularly if neutropenic, are at risk of bacterial septicemia and require hospitalization when they present with fever. Among children undergoing chemotherapy presenting with fever,

approximately 40% have a positive finding of a respiratory virus [94,95]. Even if the course of the viral infection is mild in these patients, it leads to delays of the chemotherapy treatment [93,94]. In extension, these delays may reduce the intensity and effect of the child's cancer treatment.

2.2.4.10 Other potential risk factors

Due to the cardiopulmonary, circulatory and metabolic strain of a viral LRTI, several other conditions such as severe renal disease, diabetes and metabolic diseases may decompensate with a viral LRTI [62,96].

2.3 GENERAL VIROLOGY

The ensuing chapter on general virology is summarized from Principles and Practice of Infectious Diseases, 8th Edition.

2.3.1 Virus structure

Viruses are infectious agents whose individual particles are referred to as *virions*. Virions contain the genome which is enclosed in a protein coat called *capsid*. Some virus capsids are enclosed by a lipid *envelope* with transmembrane glycoproteins which can be associated to internal *matrix proteins*. Viruses do not have their own metabolism and require the protein and genome assembly mechanisms of a living host cell to propagate. Characteristics of the viral genome, the type of capsid symmetry and the presence of an envelope are currently used for classification of viruses. Previous classifications were based on size, shape and which organ is primarily infected.

A viral genome may consist of RNA or DNA as a single (ss) or double strand (ds). Single strand genomes can be constructed in the message sense (+) or complementary to the message sense (-). Furthermore, the genome may be constructed in a linear or circular form or divided into segments. The symmetry of the virus capsid may be helical, icosahedral, spherical or complex.

2.3.2 Propagation of viruses

Viruses propagate through interactions with a living host cell in a process involving attachment, penetration, uncoating, genome replication, assembly and release. Tropism of a virus, i.e. its ability to infect and replicate in a specific tissue, is typically defined by the attachment process. Attachment can be mediated by capsid proteins or envelope glycoproteins which bind to specific host-cell receptors. Viral entry, or penetration, can take place through fusion of enveloped viruses with the cell membrane through endocytosis. Some non-enveloped viruses appear to be able to penetrate through capsid proteins capable of forming pores in the cell membrane. (+)ssRNA is required for protein synthesis from the viral genome. Hence, RNA-viruses containing dsRNA or (-)ssRNA require an RNA-dependent RNA-polymerase to synthesize (+)ssRNA intermediate to initiate protein synthesis. DNA-viruses typically utilize the cellular apparatus in the nucleus to propagate. *Inclusion bodies* are organelles created in a complex interplay between cellular and viral components in which replication of the viral genome and its proteins take place. Viral components are transported via the cellular microtubule network to virion assembly sites. During the process of viral release, enveloped viruses typically assume a part of the host-cell membrane.

2.3.3 Transmission of respiratory viruses

There are three main routes that an infected individual can transmit a respiratory virus to a susceptible individual: contact transmission, droplets and small aerosols. Contact transmission refers to direct contact with the virus carried on an infected individual or indirectly on a contaminated surface (also called fomite). Droplets are larger particles, typically defined as >10 μm, that are created during sneezing or coughing, seldom travelling further than one meter in the air. Aerosols are smaller particles, <5 μm in size, capable to remain suspended in the air for a longer time and hence travel further distances. Contact and droplet transmissions infect the upper respiratory tract, whereas aerosols can reach the lower airways as well [97].

2.4 SPECIFIC VIROLOGY

2.4.1 Influenza virus (IFV)

Influenza viruses type A, B and C (IFV-A, IFV-B, IFV-C) are part of the Orthomyxoviridae family, have a segmented genome consisting of (-)ssRNA and an envelope composed of the viral proteins and the host-cell membrane. Furthermore, IFV-A is subtyped based on antigenic properties of the envelope glycoproteins hemagglutinin (H or HA) and neuraminidase (N or NA), which are involved in viral propagation. Attachment of IFV is initiated through binding of HA to sialic acid-containing receptors on the surface of respiratory epithelial cells. NA is involved in the release of newly assembled virions by removing sialic acid from the surface of the forming viral envelope.

There is compelling evidence suggesting that IFV-A in humans has evolved from avian influenza via domesticated animals: First, all known subtypes of HA and NA are represented in IFV-A of avian species, predominantly among migratory waterfowls. Second, in contrast to human IFV-A, the avian counterpart appears to have reached evolutionary stasis based on phylogenetic studies. Apart from humans and birds, the natural host range of IFV-A encompasses swine, equine and marine mammals. IFV-B only infects humans, while IFV-C infects both humans and swine. All types of IFVs are subject to antigenic drift resulting from gradual mutations in their genome. Seasonal influenza epidemics by IFV-A and -B are driven by this mechanism as the smaller antigenic variations leads to a relative reduction of herd immunity. IFV-C has thus far only been identified in occasional episodes of upper respiratory tract infections. The propensity of IFV-A to cause pandemics results from substantial antigenic changes to either or both HA and NA. Examples of antigenic shift leading to an IFV-A pandemic was the “Spanish” H1N1 pandemic of 1918, the “Asian” H2N2 pandemic of 1957, the “Hong Kong” H3N2 pandemic of 1968 and the 2009 pandemic caused by H1N1pdm09 Influenza A (IFV-A pdm09). The last example is a result of reassortment of human and swine influenza strains within a swine population, which is why it was initially referred to as the “swine flu”. [98,99]

After infection with an influenza virus, illness starts to manifest after a short incubation period of 48 – 72 hours. Typically pronounced fever with myalgia as well as malaise and anorexia present in association with focal signs of an URTI. In most uncomplicated cases, fever subsides within four days, while respiratory symptoms may persist for weeks after primary infection. The most common respiratory complications are otitis media, obstructive bronchitis and pneumonia. Neurologic complications have been identified in 7-9% of hospitalized children. Seizures account for more than half of the neurologic manifestations, whereas encephalitis has been reported in approximately 1% of hospitalized children [60,100,101].

Influenza vaccines can be distinguished into three main types: inactivated influenza vaccines (IIVs), recombinant influenza vaccines (RIVs) and live attenuated influenza vaccines (LAIVs). LAIVs and most IIVs use an egg-based manufacturing process where the candidate virus strain is grown in eggs. Harvested viruses are inactivated in the case of IIVs or "weakened" in the case of LAIVs. Some IIVs are produced in cell-lines which has the potential of faster start-up manufacturing process. RIVs are constructed by inserting the candidate HA gene into a now recombinant baculovirus. Subsequent infection with the recombinant baculovirus in a cell-line leads to a substantial production of the candidate HA-antigen. After collection, a concentrate of the candidate HA antigen is obtained through a purification process. Depending on the amount of influenza lineages contained within a vaccine, they are called trivalent or quadrivalent. Trivalent contain two IFV-A lineages (H1N1 and H3N2) and one IFV-B lineage. Quadrivalent contain the same to IFV-A lineages as well as both IFV-B lineages currently in circulation, named Victoria and Yamagata [102–104]. Some vaccines include adjuvants which increase the immunogenicity of antigens. Adjuvants are particularly useful in a pandemic setting when large vaccine productions are needed as they require lower doses of antigens to elicit a protective immune response. In the 2009 pandemic caused by IFV-A pdm09, an adjuvanted vaccine consisting of squalene and α-tocopherol (Pandemrix, GSK) was used in several countries. Retrospective data suggest a more sustained response of the adjuvanted vaccine spanning over two years, compared to the non-adjuvanted seasonal IFV vaccines [105]. Unfortunately, an increase in narcolepsy, with a risk ratio of 5-14 among children in Finland, was detected in the ensuing two years following vaccination with Pandemrix. Partly due to the complexity of narcolepsy as a disease, a causative explanation has remained elusive [106].

Most European countries, including Sweden, recommend vaccination of children older than 6 months of age with risk factors such as: chronic cardiac and respiratory condition, conditions associated with respiratory mucus stagnation (e.g. neuromuscular conditions), chronic liver and renal disease, diabetes mellitus and conditions associated with substantial immunodeficiency [107].

Two types of antiviral agents are available for treatment against severe influenza infection: M2-inhibitors and NA-inhibitors. The viral matrix protein 2 (M2) is responsible for viral uncoating upon entry into the host cell. The two existing M2-inhibitors, Amantadine and Rimantadine, are only active against IFV-A. However, currently circulating H3N2 and H1N1

are resistant to both drugs due to point mutations in the M2-gene. As NA-inhibitors, Oseltamivir and Zanamivir interfere with the release of newly produced virions from the host cell. The drugs are active against IFV-A and -B apart from sporadic occurrences of resistant H1N1 strains [98]. Baloxavir, a viral cap-dependent endonuclease inhibitor interfering with the viral transcription process has been approved in the USA and Japan for uncomplicated influenza within 2 days of disease onset in adults and adolescents [108,109].

The onset of disease symptoms in IFV infections is also near the peak of viral replication. Therefore, antiviral treatments must be commenced within 48h after disease onset to be able to affect the course of disease. In most cases a severe course of disease is not evident until several days into infection, when the window for antiviral treatment has already passed. In addition, positive results regarding efficacy of oseltamivir treatment in children have not been confirmed in subsequent randomized controlled trials [110]. A combination of these issues may explain the decline in use of oseltamivir in clinical practice [111].

2.4.2 Respiratory Syncytial Virus (RSV)

The family Paramyxoviridae encompasses the subfamily Pneumoviridae with the two genera *Metapneumovirus* and *Pneumovirus*. *Pneumoviruses* encompass human, bovine, ovine and caprine RSV as well as murine and canine pneumovirus. The RSV genome consists of nonsegmented (-)ssRNA and the nucleocapsid is enclosed by an envelope derived from the host cell membrane. Three transmembrane glycoproteins are situated on the viral envelope: F, G and small hydrophibic protein. RSV attachment to the host cell is predominantly mediated by the G protein. The F protein enables fusion of the viral envelope to the infected host cell and its subsequent fusion to neighboring cells resulting in the characteristic syncytia.

RSV F- and G-proteins are the main antigen targets of humoral immunity. Genetic diversity is also predominantly manifested in the G-protein, which carries most of the distinguishing features between the main antigenic groups RSV-A and RSV-B. Different strains of these antigenic groups co-circulate in varying proportions during seasonal epidemics [112,113]. In tropical climates, RSV activity oscillates with relative increases related to rain periods. In temperate climates, RSV causes seasonal epidemics during the winter months and subsides during the summer months [114–117].

Infected individuals may spread RSV through large-particle aerosols in coughs or sneezes to susceptible individuals within a one-meter radius. The virus may also be contracted through contact with objects contaminated with the virus. Naturally and experimentally acquired infections have demonstrated incubation periods ranging from 2 to 8 days. The initial presentation of the disease is symptom of an URTI and subsides uneventfully for most individuals within a week. Infants, particularly those with older siblings, premature babies and young children with cardiopulmonary conditions or conditions hampering expulsion of respiratory excretions are at risk of developing severe LRTIs. Approximately 9% of hospitalized children with RSV require treatment at the pediatric intensive care unit (PICU).

Bronchiolitis cases typically show a protracted course with respiratory symptoms peaking at day 5-7. Bacterial complications are generally uncommon but typically manifest as otitis media and pneumonia. However, bacterial superinfection is often suspected in children requiring mechanical ventilation [118]. After recovery, an increased risk for wheezing in association with renewed viral RTIs is seen in children up to 11 years of age [119].

Supportive care focuses on alleviating respiratory distress, mobilizing respiratory secretions as well as upholding hydration and nutrition. The antiviral ribavirin has shown in vitro effects as well as preventing progression of URTI to LRTI and reducing all-cause mortality post HSCT as an aerosol treatment. As for most antivirals in RTIs, effectiveness depends on early initiation of treatment, i.e. within 2-3 days. However, most children develop the most severe symptoms nearly a week into the infection. Combined with the teratogenic effect of the aerosol, the clinical use of ribavirin has remained very limited [120].

RSV has been attributed to 22% of all ALRIs in children 0-5 years based on estimates for 2005, resulting in approximately 3,5 million hospital admissions[1]. It accounts for more than 60% of LRTIs in all children, primarily manifesting as bronchiolitis in infants and viral pneumonia in children older than one year of age [121]. Vaccine development against RSV was severely hampered after a vaccine trial in the 1960s containing formalin-inactivated RSV resulted in similar infection rates compared to placebo. More disturbingly, 65% of vaccinees required hospitalization with two LRTI-related deaths compared to 5% hospitalizations and no deaths in the placebo group [122]. Vaccine development is still ongoing with many vaccine candidates in phase I and phase II clinical trials, but thus far no vaccine has made it to the market [123–125]. Since the 1990s, passive immunization using a monoclonal antibody, palivizumab, specific for the viral F-protein has been available. Unfortunately, palivizumab is expensive and requires monthly administrations during RSV epidemics which limits its use to a few high risk-groups such as premature infants and infants with cardiopulmonary disease [120,126].

2.4.3 Metapneumovirus

Another member of the *Pneumoviridae* subfamily is human Metapneumovirus (MPV). It was discovered in 2001 in the Netherlands among children with clinical symptoms similar to RSV [127]. Genetic evidence suggests that the MPV diverged from avian metapneumoviruses 200 to 300 years ago [128]. Like RSV, MPV is an enveloped (-)ssRNA virus capable of syncytia formation. MPV shares all its 8 genes with RSV but lacks the genes for nonstructural proteins 1 and 2 (NS1 and NS2). Mode of transmission, epidemiology and clinical manifestations are nearly identical to RSV, apart from the peak age at admission which occurs in somewhat older children. Biennial, wintertime MPV epidemics found in temperate climates tend to occur inversely with RSV, i.e. large RSV epidemics coincide with smaller hMPV epidemics and vice versa [129,130]. This epidemiologic relationship appears intuitive given the shared target

population as well as the structural and antigenic similarities of both viruses. Ribavirin has an antiviral effect for MPV as well, but with the aforementioned limitations [131].

2.4.4 Parainfluenza virus

Parainfluenza viruses (PIV) are like the previously mentioned members of the *Paramyxoviridae* family, enveloped (-)ssRNA viruses. Of the four human serotypes PIV 1-4, PIV-1 and PIV-3 share the genus *Respirovirus*, while PIV-2 and PIV-4 belong to the genus *Rubulavirus*. The hemagglutinin-neuraminidase (HN) glycoprotein is essential for both virus attachment and release. Cell membrane fusion to the target ciliated epithelial cells in the RTI is mediated by the fusion (F) protein. Both the HN and the F protein are targets for neutralizing antibodies. PIV-3 is the most frequently identified serotype at approximately 50%, followed by PIV-1 at 26% and PIV-2 at 12%. PIV-4 is a rare finding. 50% of children have experienced a PIV-3 infection during the first year of life and by the age of 3 years the percentage infected has increased to 92%. Temperate climates typically experience biennial epidemics of PIV-1 in the fall, while PIV-3 epidemics occur annually in the spring. Viral replication peaks day 2 to 5 after infection which predominantly presents as croup in children. PIV infection may also manifest as URTIs or less commonly as LRTIs. There is no specific antiviral treatment for PIVs apart from weak evidence for ribavirin in immunocompromised patients. However, corticosteroids have an established effect as a treatment for coup. No PIV vaccine has been licensed as of yet [132,133].

2.4.5 Rhinovirus

The Rhinovirus (RV) species belongs to the family *Picornaviridae* within the genus *Enterovirus*. The virions are nonenveloped and are consist of icosahedral capsids containing four proteins (VP1-4) and the (+)ssRNA genome. RV antigenic diversity is primarily found in VP1-3 with VP1 responsible for the attachment process to host cells. There are more than 100 RV serotypes with a “major” RV serotype group containing 90% of known serotypes that attach to intracellular adhesion molecule 1, while the “minor group” utilizes low-density lipoprotein receptor for attachment. In contrast to other respiratory viruses and closely related EVs, most RVs are thermostable, remaining infective on surfaces for hours to days on environmental surfaces up to +37°C and for years at -70°C.

Children are infected at least once a year, though approximately one fourth of RV infections remain asymptomatic in children under 4 years [134]. The virus is transmitted through direct contact or through or aerosols. Symptomatic infections become apparent after an incubation period of 2 days. RV infections are found throughout the world causing one-half to two-thirds of common colds. Apart from URTIs including otitis media and rhinosinusitis, RV infections can lead to croup, bronchiolitis, bronchitis and pneumonia. The capsid binding agent pleconaril has shown moderate effect regarding duration of illness but has not been licensed

for RV-infections. Given the number of serotypes, vaccine development has been challenging and no RV vaccine has been evaluated in a clinical trial [135–137].

2.4.6 Enterovirus

Sharing the genus *Enterovirus* with RV means that the main viral structures are similar to RVs. Human Enteroviruses (EVs) distinguish themselves by their ability to remain infective after exposure to a wide range of pH (from 3 to 10). Its propensity to infect the gastrointestinal tract results from EVs' ability to avoid degradation in the acidic gastric environment and has rendered the viruses name. Human EVs consist of four species (A-D) and more than 100 serotypes. EVs may be shed in feces or in respiratory secretions and are predominantly transmitted through direct contact. EVs propagate in submucosal lymphatic tissues of the respiratory or gastrointestinal tract inducing URTI and gastrointestinal symptoms. EVs may spread from the mucosa to regional lymph nodes from where a transient "minor viremia" may occur. Subsequent dissemination to reticuloendothelial tissues in the liver, spleen and bone marrow. If the virus is not controlled at this stage, a "major viremia" associated by fever, leads to dissemination to the central nervous system, heart and skin. Apart from the more common presentation of respiratory and gastrointestinal infections, EVs may cause aseptic meningitis, encephalitis, myocarditis and exanthemas and "hand foot and mouth disease". In monthly sampling of Norwegian infants from 3 months to 28 months of age identified EV infections among 51% of the children, underscoring the high prevalence of the virus [138]. Seasonal peaks occur during the summer months in temperate climates [139]. There is limited evidence suggesting clinical benefit of the aforementioned capsid antagonist pleconaril, but more studies are needed [140–142]. A vaccine against the EV serotype EV-71, which is known to cause hand, foot and mouth disease has been licensed in China [143].

2.4.7 Coronavirus

The Coronavirus (CoV) family encompasses the genus *Alpha-, Beta-, Gamma- and Deltacoronavirus* of which the first two are relevant to humans. Human coronavirus 229E and NL63 belong to the genus *Alphacoronavirus*. The genus *Betacoronavirus* includes CoV-OC43, CoV-HKU1, severe acute respiratory syndrome CoV (SARS-CoV-1) as well as middle east respiratory syndrome (MERS) CoV and the recently emerged SARS-CoV-2 responsible for Covid-19 disease [144].

CoV virions are enveloped and contain a (+)ssRNA genome within its helical nucleocapsid. The spike (S) glycoprotein with its club-like formation on the surface of the viral envelope produces the crown-shape in the microscope which gave the virus its name. The S protein consists of two functional subunits responsible for receptor binding (S1, bulb) and membrane fusion (S2, stalk). Specific surface enzymes of host cells function as receptors for the S1

protein, which is the angiotensin converting enzyme 2 (ACE2) in the case of CoV-NL63 and SARS-CoV-1 and -2 [145].

The established human CoVs 229E, HKU1, NL63 and OC43 are often asymptomatic or cause mild URTIs. Rarely, the CoVs are found in hospitalized children with LRTIs and in half of those cases another, more pathogenic virus such as IFV or RSV is found in the same sample. During the 2002 – 2003 outbreak of SARS-CoV-1, children accounted for less than 2% of all cases and only 5% of all pediatric cases required hospital admission. The disease was both milder and less infectious in children and no pediatric deaths have been reported. Children typically presented with fever, cough and nausea. Children older than 12 years were more likely to develop LRTI in the second week of disease similar to disease presentation in adults. Since the initial reports from Saudi Arabia in 2012, MERS-CoV has occurred sporadically. Among 14 confirmed cases, 9 were asymptomatic and detected by screening of household contacts. Three developed mild URTIs, while two children with underlying conditions progressed to severe LRTI with subsequent multi-organ failure and death [146].

An initial cluster of pneumonia cases in Wuhan, China in December 2019 was found to be caused by the novel coronavirus SARS-CoV-2. Disease severity in children was presented in a meta-analysis of predominantly Chinese, confirmed pediatric Covid-19 cases found 20% to be asymptomatic, 33% mildly, 51% moderately, 7% severely and 5% critically ill [6]. In addition, a rare post-infectious inflammatory response condition labeled multisystem inflammatory syndrome has been observed in children. The syndrome tends to present in older children with a median age at 8,6 years and severe illness necessitating PICU admission in 68% of cases [147,148].

Treatment of infections of CoVs in children is symptomatic. Several types of vaccines against SARS-CoV-2 have been developed based on inactivated virus, viral subunits, mRNA and vector-based vaccines [149]. The mRNA vaccine Tozinameran (Comirnaty, Pfizer BioNTech) has recently been approved in Sweden for children from 12 years of age.

2.4.8 Adenovirus

Adenoviruses (AdVs) are nonenveloped, dsDNA viruses with an icosahedral viral capsid of which all species found in mammals belong to the genus *Mastadenovirus* of the *Adenoviridae* family. More than 50 serotypes and 70 genotypes have been identified and categorized into 7 species (AdV-A to AdV-G). Tissue tropism and subsequent clinical manifestations have been related to serotypes as well as site of viral entry. AdV-A, -B, -C and -E preferably replicate in the respiratory tract. The gastrointestinal tract may also be infected by AdV-A as well as AdV-D. AdVs of the group D are implicated in infections of the conjunctiva [150]. 17% of children admitted with a confirmed viral RTI during an eight-year period in Spain had an AdV infection. Less than half of these were single-AdV infections (38%) and the clinical courses of co-infections tended to be more characteristic of the other virus, namely as RSV, IFV and RV [151]. AdV is also recognized as an opportunistic infection in severely

immunocompromised children, i.e. post HSCT, SOT and acquired immune deficiency syndrome. The antiviral cidofovir has been associated with clinical improvements in some studies, but also with significant nephrotoxicity [152]. In general, only supportive care is motivated in children with AdV infections. Oral live vaccines for ADV types 4 and 7 have been administered in the US military since 1971. During a 12-year discontinuation of AdV vaccinations from 1999 to 2011 several AdV-outbreaks, with up to 80% of recruits infected, emerged [153].

2.4.9 Bocavirus

Bocavirus (BoV) was first described in 2005 and has putatively been allocated to the family of *Parvoviridae*, subfamily *Parvovirinae* and the genus *Bocavirus*. It is a nonenveloped virus with an isometric nucleocapsid containing the ssDNA genome [154,155]. In a meta-analysis of children <2 years with bronchiolitis, BoV was identified as the sole infectious agent in 4% and as a co-infection in 8%. In a study of community-acquired pneumonia of children <5 years, BoV was identified nearly twice as often in healthy controls [5]. An important challenge to establishing a clear clinical role of BoV is related to the long duration of viral shedding [155].

2.5 IMMUNE RESPONSES TO VIRAL RESPIRATORY TRACT INFECTIONS

2.5.1 General aspects of immune responses

The respiratory tract must maintain a delicate balancing act in limiting potential damages caused by environmental exposures and microbial agents while sustaining the vital function of gas exchange. In this respect, the adult respiratory tract benefits from a mature organ, an established commensal microbiota, immune cells highly responsive to infections as well as specific immune memory adapted to past experiences of microbial infections. Conversely, the newborn lung is still under development and its respiratory tract is in the process of acquiring its commensal microbiota. Newborn immune cells are both tasked with the process of organ maturation and adapted to a less responsive state suited for life in utero. Apart from maternal antibodies, the newborn immune system also lacks specific immune responses that develop following previous infections [156].

The epithelium of the respiratory tract forms the initial barrier to external exposures. It forms a physical layer strengthened by intracellular connections such as tight junctions. Mucous produced by the epithelium contains peptide antibiotics that provide a first line of microbial defense. Intraepithelial lymphocytes recognize microbe antigens, however with limited diversity. Tissue resident alveolar macrophages can be activated to initiate an inflammatory response, ingesting microbes and further antigen presentation or alternatively engage in tissue repair and dampening inflammation. Activated dendritic cells both contribute to the innate immune responses such as cytokine and interferon production as well as mobilizing adaptive cellular and humoral responses by the adaptive immune system. Interferons induce cellular expression of enzymes with capacity to degrade viral RNA, inhibiting viral gene expression and protein translation as well as virion assembly [24].

Initial recognition of a viral infection is mediated by cellular pattern recognition receptors (PRRs) capable of binding pathogen-associated molecular patterns. PRRs are located on the cell surface, in endosomes and in the cytosol in respiratory epithelial cells, fibroblasts and antigen-presenting cells (APCs) of the respiratory tract but with different expression patterns. Upon activation, PRRs initiate downstream cellular transcription factors inducing expression of interferons and chemokines, apoptosis of the infected cell as well as changed expression of PRRs [157].

2.5.2 The role of Prostaglandin E_2

The prostaglandin E_2 (PGE_2) molecule has a modulating role in various tissues and organs spanning from central regulation of body temperature, neuronal signaling, several kidney functions, smooth muscle control as well as immune responses [158–162]. Its effects are both determined by expression of various prostaglandin E receptors, coupled signaling pathways,

tissue and cell type [163]. PGE_2 has a complex role in inflammatory responses, but with a net effect of tissue preservation through inhibition of cytotoxic type 1 immunity, attracting neutrophils and macrophages while promoting them to engage in tissue repair [162,164]. PGE_2 synthesis is initiated by phospholipase A_2-mediated release of arachidonic acid from the cell membrane. Subsequently cyclooxygenases (COXs) convert arachidonic acid into prostaglandin H_2. COX1 is a constitutively active cyclooxygenase, whereas COX2 is inducible. Prostaglandin H_2 conversion into the biologically active form PGE_2 is mediated by the prostaglandin E synthases. In vivo degradation of PGE_2 is rapid and controlled by 15-hydroxyprostaglandin dehydrogenase, which makes reliable sampling of systemic levels challenging. The final product of PGE_2 metabolism, the tetranor PGE metabolite found in the urine, is considered to best reflect systemic PGE_2 levels [165].

2.5.3 Immune evasion by respiratory viruses

The coevolution of microbes and the human immune system is often described as an arms race. In this regard, viruses have developed mechanisms to avoid, subvert and divert the human immune responses. Viral genomes, RNA-genomes in particular, often have a high mutational rate which allows the virus to avoid trained responses from the adaptive immune system in previously infected individuals [166,167]. As also mentioned in chapters for specific viruses, the highest genetic diversity of the viral genome is typically found in attachment glycoproteins which are also the most common target of neutralizing antibodies. The ability of RSV and MPV to induce syncytia with adjacent cells reduces the risk of exposure to immune cells and PRRs in the process of viral attachment and entry. The potency of the type I interferon (IFN) system for viral defense is underscored by the fact that all respiratory viruses have mechanisms to avoid the type I IFN response [168,169]. In the case of RSV and IFV, this ability has been shown to determine the viral host range [170–172]. RSV infections have also been associated with increased levels of IL-10 and PGE2 which stimulate tolerogenic rather than cytotoxic immune responses hampering the host's ability to clear the infection and develop protective immunity [173–175].

2.5.4 Immunopathology of viral infections

Understanding the main driver of pathology in respiratory viral infections can have important therapeutic implications. If the disease is caused by direct viral damage, antiviral treatment and perhaps even immune-enhancing treatments should have the most potent effect. However, if disease is mainly driven by an excessive inflammatory response, immune-suppressive treatments are indicated. On the one hand, high viral loads have been associated with increased disease severity for RSV, IFV and MPV [176–178]. On the other hand, HIV-positive infants infected with RSV shed high levels of RSV while developing bronchitis to a lesser extent [179]. The aforementioned disastrous result of the RSV vaccine trial in the 1960s also supports the notion that immunopathology is important for development of severe

disease. Furthermore, RSV bronchiolitis disease severity typically peaks around 6th day of infection when the adaptive immune responses are expected to contribute to infection control which is well beyond the therapeutic window of antiviral agents. Discerning the role of viral damages and immune response is challenging since high infection with higher viral loads leads to a more pronounced infection and subsequently to a greater immune response. In this regard, the contribution of viral load and immunopathology to disease severity are perhaps not mutually exclusive theories, but rather a matter of timing.

2.6 CONDITIONS FOR VIRAL EPIDEMICS

Typically, drivers of viral respiratory tract infections focus on three major aspects: meteorologic and climate factors, population and host factors as well as viral factors. In practice, all these elements are intertwined. For example, meteorological conditions have a different impact on the stability of various viruses depending on the viral structure. Meteorological and climate conditions may also affect a population's behavior regarding frequency and proximity of contacts. The immune experience of a population, also called 'herd immunity', further determines the epidemic potential of a specific viral strain.

2.6.1 Meteorological and climate factors

As mentioned previously, seasonal variations of viral respiratory tract infections are the most pronounced in temperate climates with epidemic peaks occurring in winter, particularly in the case of IFV, RSV, MPV and CoV. Even though indoor temperatures are often kept at the same level, the relative indoor humidity is affected by the absolute humidity outdoors, which in turn is related to outdoor temperatures [180,181]. Relative humidity predominantly affects aerosol transmission. Virus stability and viability is best maintained at either high (60-100%) or low (10-40%) relative humidity (**Table 1**). Winter viruses appear to be most stable at lower levels of relative humidity while the opposite is true for summer viruses (e.g. EVs) [182].

Climate: season	**Absolute humidity outdoors**	**Relative humidity indoors (%)**	**Proportion of aerosols**	**Virus stability and viability**	**Mode of transmission**
Tropical: all seasons	High	60 – 100	Low	High	Fomite, direct and indirect contact
Temperate: spring/fall	Intermediate	40 – 60	Low	Low	All modes reduced
Temperate: winter	Low	10 – 40	High	High	Predominantly airborne

Table 1. Impact of climate and meteorologic factors on virus stability and predominant mode of transmission. Modified from Moriyama et al (2020) [182].

From a host perspective, physical airway barriers of the respiratory epithelium are also affected by temperature and humidity. Cold and dry air has been shown to damage airway epithelial integrity, increase mucous viscosity and decrease ciliary movement resulting in mucous stagnation [183–185]. In addition, low temperature has been shown to reduce the

interferon response in the URT, while lower Vitamin D levels associated with the darker winter months negatively affects macrophage responses [186,187].

2.6.2 Population factors

Previous chapters have emphasized the importance of age for viral susceptibility. Hence, the age-composition of a population has important implications for patterns of viral epidemics. Mathematical models of RSV epidemics have been able to reproduce observed epidemic patterns based on birth rates and environmental factors [188–191]. These models use differential equations to calculate the number of susceptible (S), infected (I), recovered (R) and yet again susceptible, hence it is called SIRS-model (**Figure 6**).

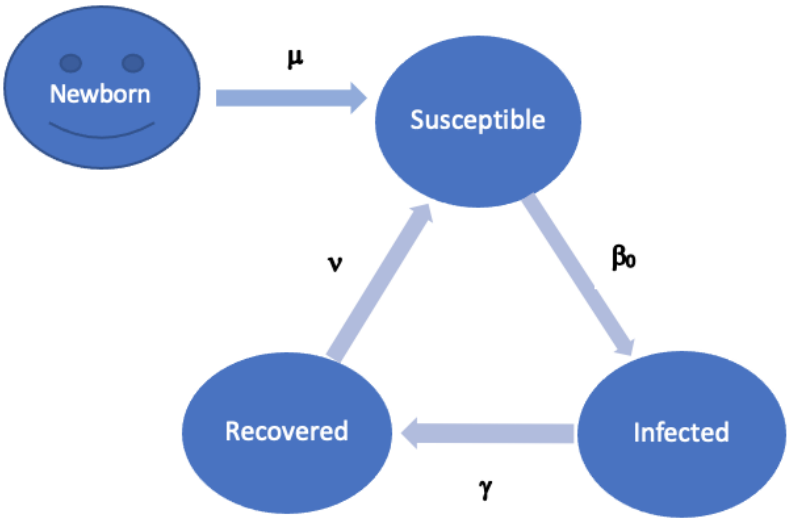

Figure 6: Newborns enter the pool of susceptible at a rate defined by birth rates (μ). Susceptible individuals become infected when in contact with infected individuals, who transmit the infection to an extent defined by the transmission rate (β_0). γ defines the rate of recovery from infection and thereby also infectiousness. Recovered individuals become susceptible again as defined by the immunity period (ν).

More advanced SIRS-models have built-in assumptions for decreased susceptibility for each acquired infection and with age. Individuals "leave" this model at the populations rate of death if not constructed to allow individuals to "outgrow" susceptibility (not shown on the graph). To simulate variations in transmission rates depending on seasonal, meteorological variations, a common practice is to add a sinusoidal function to β_0.

The role of crowding, socioeconomics and pollution are important population factors as mentioned in the chapter on risk factors for viral RTIs in children. The potential effects of both crowding and migration have been highlighted by recent policies to prevent the transmission of SARS-CoV-2 through lockdowns, as well as closed borders, schools and offices [192,193].

2.6.3 Viral factors

The extent and pace at which a respiratory virus elicits (severe) symptoms has important implications for viral transmission. An infective, but pre- or asymptomatic individual is more likely to have multiple interactions with potentially susceptible individuals than a severely ill individual. Asymptomatic infections appear to be a more common occurrence with SARS-CoV-2, particularly in children, in comparison to IFV and RSV [194–196]. Additionally, the tropism within the respiratory tract of a virus has relevance for the transmission rate as viral replication in the URT will result in higher infectiousness compared to the LRT as noted for avian influenza [197]. Clearly, the aforementioned immune evasion mechanisms are important for the capacity to spread within and later reinfect a population. Furthermore, zoonotic tropism is another viral factor of increasing importance, particularly with respect to IFV and CoV (see previous chapter on specific viruses). In this regard, animals can serve as reservoirs for already established human viruses, produce 'spill-over' infections or function as 'mixing vessels' for new viral epidemics in humans [198]. Consequently, environmental, animal health and ethics align with public health interests to sustain biodiversity, reduce the size of livestock as well as assuring animal welfare.

2.7 VIRUS-VIRUS INTERACTIONS

When studying viral interactions such as co-infections and viral interference, it is important to consider the studied age group, as attack-rates for different viruses may vary in this regard. Furthermore, seasonal patterns of the studied viruses must be known to determine to what extent co-infections and co-circulation should be expected.

2.7.1 Viral co-infections

Viral co-infections are common in both symptomatic and asymptomatic children [5]. Larger studies including >500 children spanning over several years based on hospital admissions have found viral co-infections in 17-20% [199,200]. There have been conflicting reports regarding the effect on disease severity of viral co-infections in children, but a meta-analysis by Lim et al in 2016 found no significant differences compared to single infections [201]. Viral co-infections are more often found in children at the age when they enter pre-school, while single infections are more common in infants [202].

2.7.2 Viral interference

The case for viral interference has been proposed in several epidemiological studies, which suggest that the spread of one virus inhibits another [203–206].The broadest definition of the term viral interference is the inhibitory effect of one virus on the spread of the other. This inhibitory effect can occur on a host level, where presumably the upregulated interferon system following a viral infection, inhibits infections by a second virus as has been shown for IFV and MPV in a ferret model [207]. Viral interference can also occur on a behavioral level as an individual who has succumbed to one infection is more likely to stay at home, consequently reducing exposure to other viruses. In a broad perspective, public health policies instated in order to prevent or dampen a pandemic, as in the case of SARS-CoV-2, can also inhibit the spread of seasonal viruses [208]. In addition to the diminished circulation of IFV during the winter of 2020-2021 dominated by SARS-CoV-2 [209], also RSV levels remained exceptionally low [209,210]. Most likely due to loss of herd immunity in the “lost” RSV winter epidemic, several countries report an early surge in RSV cases which started in August/September 2021 [210,211]. Reports regarding possible viral interference between RVs and the emerging H1N1pdm09 Influenza A virus (IFV-A pdm09) inspired the research question in study I [212–214].

2.7.3 Viral synergism

Based on knowledge regarding immune evasion properties, it is also conceivable that a viral RTI can 'pave the way' for a subsequent viral infection by subverting the immune response [215]. Coronaviruses provide an interesting example with contradictory effects of interferon-inducible transmembrane proteins (IFITMs). These IFITMs are capable of disrupting viral entry of CoV-NL63, CoV-229E, SARS-CoV-1 and MERS-CoV. On the other hand, CoV-OC43 exploits IFITMs to expedite its entry [145]. With this in mind, its notable that viral findings among hospitalized children 2008 – 2016 with CoV at our hospital suggest co-circulation of CoV-OC43 with CoV-NL63 in a biennial pattern (manuscript in preparation).

3 RESEARCH AIMS

The general aims of this thesis are to advance the knowledge regarding the burden and risk-factors of severe RTIs in children caused by IFV and RSV, to identify drivers of viral respiratory epidemics and to add a diagnostic tool for severe viral RTIs. This knowledge can be applied to improving prevention methods, healthcare resource planning and to identify individuals at risk of progressing to severe RTIs.

Disease burden, epidemic pattern, risk factors and complications were investigated in **studies III-V**. The role of specific epidemic drivers, with an emphasis on viral interference in study I, were inquired in **study I and V**. **Study II** investigated the diagnostic potential of urinary prostaglandin E_2 metabolite in infected infants.

*3.1.1.1 **Study I:** Respiratory viruses in hospitalized children with influenza-like illness during the H1N1 2009 pandemic in Sweden*

We inquired for evidence of viral interference between RVs and the new pandemic IFV (H1N1pdm09 Influenza A) based on viral findings in hospitalized children in Stockholm between 1st July and 30th December 2009.

*3.1.1.2 **Study II:** Urinary PGE_2 metabolite levels in hospitalized infants with infections compared to age-matched controls*

The aim of the study was to establish baseline levels of the urinary PGE_2 metabolite in healthy infants and to compare them to age-matched infants hospitalized with respiratory or gastrointestinal infections.

*3.1.1.3 **Study III:** Influenza epidemiology among hospitalized children in Stockholm, Sweden 1998-2014*

The objective of this study was to provide comprehensive data from children hospitalized with influenza with respect to IFV type, underlying conditions and complications as a base for vaccine recommendations in children.

*3.1.1.4 **Study IV:** Rates and risk factors of severe respiratory syncytial virus infection in 2008-2016 compared with 1986-1998*

The purpose of this inquiry was to find possible changes in the epidemic pattern in children admitted with RSV at least one decade apart, after introduction of more sensitive diagnostic measures, targeted prevention with passive immunization and possibly other therapeutic improvements.

*3.1.1.5 **Study V:** Exception makes the rule: Disruption of the delayed biennial pattern of respiratory syncytial virus epidemics in Stockholm (manuscript)*

The objective of this study was to identify factors that could have influenced the temporary change in the seasonal RSV epidemic pattern in Stockholm during the two winters from 2009 to 2011.

4 MATERIALS AND METHODS

4.1 STUDY DESIGNS

Studies I, III-V were all retrospective epidemiological studies. In studies III-V, spanning over the periods 1998-2014, 2008-2016 and 1998-2016 respectively, data collection was conducted on an annual basis. Study II was designed as a prospective, cross-sectional study with age-matched controls.

4.2 PATIENTS

Except for study II, all studies included in the thesis are based on hospitalized children living in the catchment area of Astrid Lindgren Children's Hospital in Solna, corresponding to the northern Stockholm region. For each case, virological results were compared to medical records to ascertain that the child had been admitted with clinical signs of a viral RTI at the time of sampling. Also, and discharge diagnosis code of RSV and IFV was double checked with virological results. For IFV admissions from 1st July 2016 to 30th of June 2019, cases are included based on an IFV discharge diagnosis, but have not yet been confirmed with the virological database. Residency within the catchment area was determined by the child's address stated in the medical journal at the time of data collection. Risk factors, length of stay and PICU admission were also retrieved from medical records.

In the case of study II, primary residency in the hospital's catchment area was not required and all types of infections were included. As a definition for viral infection, a clinical diagnosis at the discretion of the treating physician was sufficient, i.e. laboratory confirmation was not required. In addition to risk factors, length of stay and PICU-admission, pharmacological treatment, type of respiratory support and laboratory results were retrieved from medical records. Furthermore, infants visiting the children's health center (Swedish: Barnavårdscentral) in Mörby Centrum in Stockholm were recruited as healthy controls in study II.

4.3 VIRUS DETECTION METHODS

4.3.1 Sampling methods

The essential inclusion criteria for studies I, III-V are microbiologically confirmed RTI infection. Samples were either collected at the pediatric emergency department or after admission to hospital wards. Respiratory samples were predominantly obtained from nasopharyngeal aspirates (approximately 75%) and to a smaller extent using nasopharyngeal swabs (approximately 10%). The remaining samples were mostly collected from tracheal secretions, while a few originated from bronchoalveolar lavage or oropharyngeal secretions. Apart from rapid antigen testing for RSV, sample analysis was conducted at the accredited

Microbiological Laboratory at the Karolinska University Hospital. However, rapid antigen testing at the pediatric emergency ward was subject to repeated quality controls by the microbiology department.

4.3.2 Immunofluorescence and virus isolation

In the period 1979-2006, all nasopharyngeal aspirates collected from Astrid Lindgren Children's Hospital during winter months were analyzed for RSV, IFV-A and IFV-B using direct a direct immunofluorescence assay. In most cases, IF results were subsequently confirmed using virus isolation methods with cell cultures given sufficient sample volume. The rest of the year, samples were analyzed using virus isolation methods [8].

4.3.3 Polymerase chain reaction

From 1st October 2007 and onward, respiratory samples were analyzed using polymerase chain reaction following sample preparation and nucleic acid extraction in an automated pipetting process. Apart from RSV-A with RSV-B, PIV-1 with PIV-3 and PIV-2 with CoV-229E, assays were performed using single-agent PCR to avoid target competition. DNA-virus assays conducted using real-time PCR, whereas RNA-virus assays included a one-step real-time reverse transcription reaction. During winter months, initial analysis included RSV-A, RSV-B, IFV-A and IFV-B ("small PCR package"). Samples negative for RSV and IFV or if prompted by the referring clinician, further analysis was performed targeting: AdV, BoV, four CoVs (229E, HKU1, NL63, OC43), EV, MPV, PIVs 1-3 and RV ("large PCR package"). Exceptionally, from 1st July 2009 unto 30th December 2009, all admitted children with any sign of infection, including seizures and apneas, were tested using the "small PCR package" which henceforth was expanded to contain primers for IFV-A pdm09.

4.3.4 Rapid antigen testing

During RSV-peaks, as confirmed by a significant rise in positive PCR-findings, rapid antigen testing was performed for RSV at the pediatric emergency ward. The test was used for children with signs of respiratory tract infections requiring hospital admission to allow cohort care and reduce the risk for nosocomial infections. The rapid antigen test BD Directgen EZ RSV was used according to the manufacturer's instruction to this end. Negative cases were retested on the wards with PCR-methods as described above. Rapid antigen testing was introduced concomitantly with PCR in 2007. Starting 2016, all RSV samples were analyzed with PCR as the analysis became available at the Karolinska University Hospital around the clock.

4.3.5 Definition of risk factors

Age at sampling, sex and underlying medical conditions were retrieved from medical journals as previously mentioned. Medical conditions were grouped as follows:

Neonatal complications: Prematurity (<37 weeks of gestational age,) requirement of postnatal surgery (e.g. gastroschisis) and other causes requiring treatment at the neonatal inessive care unit.

Chronic lung diseases: Preterm birth and need of supplemental oxygen at 36 weeks of gestational age as well as ongoing regular treatment for respiratory symptoms 6 months prior to hospitalization. In addition, malformations resulting in severe respiratory impairment, such as tracheoesophageal fistula or diaphragmatic hernia, or chronic lung diseases such as cystic fibrosis were included in this group.

Neuromuscular conditions: This group consists of children with any condition associated with impaired neuromuscular development regardless of cause, eg genetic diseases, muscular diseases, metabolic disorders, birth defects or sustained brain injury. Children with epilepsy were also included in this group.

Cardiovascular conditions: These mainly consist of children with cardiac malformations, pulmonary hypertension, but any condition with hemodynamic effects on cardiac performance was considered.

Conditions with immunosuppression: Children with oncologic conditions requiring immunosuppressive treatments, inborn immune defects, certain hematological disorders, such as sickle cell anemia, were allocated to this group.

Asthma and recurrent wheeze: An asthma diagnosis was used as defined by previous caregivers. Recurrent wheeze was defined as at least two previous anamnestic episodes of wheeze or two hospitalizations due to wheezing in children less than two years of age.

Other conditions: Many other underlying conditions were rare in the context of viral RTIs requiring hospital admission, however not necessarily uncommon in the population, were mentioned as a separate group.

For children with multiple conditions fitting into different groups, all conditions were noted with an order of priority depending on the assessed impact of the condition on the course of the child's RTI. If their importance was indistinguishable, both conditions were counted. Hence, the total number of conditions may exceed the total number of children.

4.3.6 Disease severity and complications

Length of stay, i.e. days admitted to the hospital, and admission to the PICU were used as markers for disease severity. Complications were generally categorized into three groups:

Respiratory complications: These included sinusitis, tracheitis and bacterial pneumonia as diagnosed by the treating physician. All children diagnosed with a bacterial pneumonia had been examined with a chest x-ray and had a CRP of at least 40mg/L. Otitis media was not considered a focal complication.

Neurologic complications: Both febrile and afebrile seizures were counted. In children with a preexisting diagnosis of epilepsy, seizures were counted if their manifestations were increased in frequency or severity prior to or during hospital admission. Diagnosis of encephalitis required supportive evidence from an encephalogram.

Other complications: These included other rare complications, such as myositis, myocarditis and decompensation of other underlying conditions such as acute renal failure in children with kidney disease or metabolic crisis in children with metabolic diseases. Dehydration due to feeding difficulties was not considered a complication.

4.3.7 Sample collection and analysis in study II

4.3.7.1 Urinary sampling and storage

Urinary samples were obtained through collection with a sterile gauze placed in the diaper, urinary bags or via direct sampling. In the case of sampling with a gauze, urine was retrieved by squeezing the cloth within a sterile 10 mL syringe. Samples contaminated with feces were discarded. A minimum volume of 50 μL was required for subsequent analysis. As soon as the sample was collected or provided by caregivers, it was stored at -20°C for up to one week. Subsequently, samples were split into a maximum 200 μL per aliquot and stored at -80°C until further analysis.

4.3.7.2 Analysis of the urinary tetranor metabolite of PGE_2 (u-tPGEM)

Analysis of u-tPGEM was performed with a previously developed method using liquid chromatography and detection by tandem mass spectrometry (LC-MS/MS). Urinary creatinine concentrations were measured using a commercially available creatinine assay kit based on spectrophotometry. All assays were performed in duplicates. In case of insufficient sample volume, a 1:2 dilution was used. The validity of this method using the aforementioned sampling techniques in a clinical setting had been tested prior to this study [216]. Urinary creatinine was compared to body surface as a normalizing parameter of u-tPGEM and the former was deemed more appropriate to correct for varying hydration status. Subsequent data analyses were performed using u-tPGEM normalized to urinary creatinine (u-tPGEM/crea).

4.3.8 External data

Population data for children residing in the catchment area as well as birth rates were attained from online databases provided by Statistics Sweden and the Swedish National Board of

Health and Welfare respectively. Temperature data were obtained from the Swedish Meteorological and Hydrological Institute's online database. Data on childcare enrollment was acquired from the online database provided by the Swedish National Agency for Education.

4.3.9 Statistical analysis

Results from continuous variables obtained in the **studies I-IV**, such as age, length of stay and u-tPGEM levels, did not have a gaussian distribution. These results were described using the median and interquartile ranges (IQR) and a nonparametric test was used (Mann-Whitney U test) for statistical analysis. Confidence intervals were tested using exact Clopper Pearson binominal confidence intervals. In **study II**, correlation between the continuous, non-gaussian distributed, variables such as age and u-tPGEM/crea were analyzed using Spearman's correlation analysis. A multivariate analysis was used in **study III** with IFV type as the dependent variable and age, risk factors as well as complications were independent variables. To this end, a generalized linear model constructed by the logit function in Statistica® was applied. A *p*-value less than 0.05 was considered statistically significant.

4.3.10 Ethical considerations

4.3.10.1 General considerations

As previously mentioned, **studies I, III-V** were based on retrospectively collected data requiring access to each included child's medical record. Results were only presented on a group level and therefore no identifiable data can be linked to a specific individual. The complete data set has only been accessible to persons involved in the specific studies. Given that the potential harm of the temporary infringement of the child's privacy is limited, the long timespan covered by several studies and the number of participants involved, it was not feasible to seek approval of participation for each individual.

In **study II**, for all included infants at the hospital and control cases at the children's health center an informed consent was signed by at least one parent after oral and written information had been given. Care was given to avoid additional strain on infants and their parents in hospital cases. When a significant language barrier was evident between investigators and the infant's parents, inclusion into the study was not sought for. The sampling technique using gauze pads was used to minimize harm and discomfort to the infants. As in the aforementioned studies, results were only presented on a group level and no identifiable data can be linked to a specific individual. Access to the data set was limited to persons involved in the research.

All studies included in this thesis have been reviewed and approved by the Swedish Ethical Review Authority.

4.3.10.2 Registration numbers for ethical approvals per study

Study I	Dnr 2009/1878-31
Study II	Dnr 2011/1908-31
Study III	Dnr 2009/1878-31
Study IV	Dnr 2009/1878-31, Dnr 2017/1918-31
Study V	Dnr 2017/1918-31

5 RESULTS AND DISCUSSION

There are overlapping directions of inquiry in the studies included in this thesis. For instance, in studies I and V regarding viral interference and potential drivers of viral epidemics and regarding disease manifestation and epidemiology in studies III and IV. For didactic reasons, results will be presented and discussed in a thematic manner.

5.1 URINARY PGE_2 METABOLITE AS A POTENTIAL MARKER FOR VIRAL INFECTION IN CHILDREN (STUDY II)

5.1.1 Rationale for investigating PGE_2

As mentioned in the introduction on immunological responses to viral infections, PGE_2 has a role in directing the immune response to protect the respiratory epithelium from excessive inflammation, which is important for the preservation of organ function [217,218]. On the other hand, viruses may stimulate PGE_2 as an immune evasion mechanism. Indeed, inhibition of PGE_2 *in vitro* has shown improved antiviral responses by macrophages [219]. *In vitro* studies on RSV infection of epithelial cells, using the A549 cell line (lung carcinoma cell), showed increased PGE_2 levels, which in turn increased the rate of viral replication [220]. However, increased PGE_2 secretion is also an immune evasion mechanism shared by cancer cells, including A549 cells, therefore the mentioned results generated in these cells should be interpreted with some caution [221]. *In vivo*, tracheal aspirates collected from infants with severe RSV infections had markedly higher PGE_2 levels compared to age matched, uninfected infants undergoing anesthesia for elective procedures [222]. In a mouse model, inhibition of PGE_2 significantly improved the outcome of IFV-A infection [223]. Even though PGE_2 was not specifically studied, the general shift towards a tolerogenic immune response has been shown for other respiratory viruses both *in vitro* and in infants [224,225]. PGE_2 may also have a role in viral gastroenteritis as elevated levels have been associated with rotavirus infection, and inhibition of cyclooxygenases resulted in reduced viral replication [226,227].

The rationale for focusing our investigation on infants was that they have the highest risk of developing severe viral disease. In addition, *in vitro* and *in vivo* data suggest, that infection induced increases of PGE_2 in the central nervous system can lead to depression of central regulation of respiration resulting in apneas in infants [228–230]. Apneas are often observed in newborns and preterm infants in association with infections [231]. Thus, advancing the knowledge of PGE_2 levels in infected infants could have implications for our understanding of apneas as well [232].

Knowledge of PGE_2 levels in infants is scarce, partly due to the need of invasive sampling and the advanced analysis methods required [233,234]. Therefore, access to a feasible and non-invasive method to measure u-tPGEM, which best reflects systemic PGE_2 levels was an important prerequisite for this study [165,216].

5.1.2 Subject inclusion, sampling, u-tPGEM analysis and clinical categorizations

Control subjects were included concomitantly with hospital cases during two consecutive winters (December – March, 2011-12 and 2012-13). A total of 230 samples were successfully analyzed from 81 healthy infants and 142 infants hospitalized with and infection. An additional 37 samples had been collected, but a reliable analysis result was not achieved due to technical issues, such as lack of volume or unreliable results of positive controls. 4 healthy controls were excluded due to symptoms of URTI or recent intake of acetaminophen or ibuprofen. Cases were allocated to diagnosis groups, with three groups consisting of at least 10 cases: viral URTI, viral LRTI and viral gastroenteritis (GE) (**Figure 7**). Viral LRTIs included obstructive bronchitis, bronchiolitis and viral pneumonia due to the inherent challenges in differentiating between the three as mentioned in the introduction. The most dominant viral finding was RSV, consisting of 68% of LRTIs. For GE cases, rotavirus was identified in 7/8 positive cases. Diagnosis groups consisting of fewer than 10 cases were deemed too small for between group comparisons. 87% of samples from hospitalized infants were obtained within a week of disease onset (median 4, IQR 3-5 days). Characteristics of the cohort are also presented in **Table 2**.

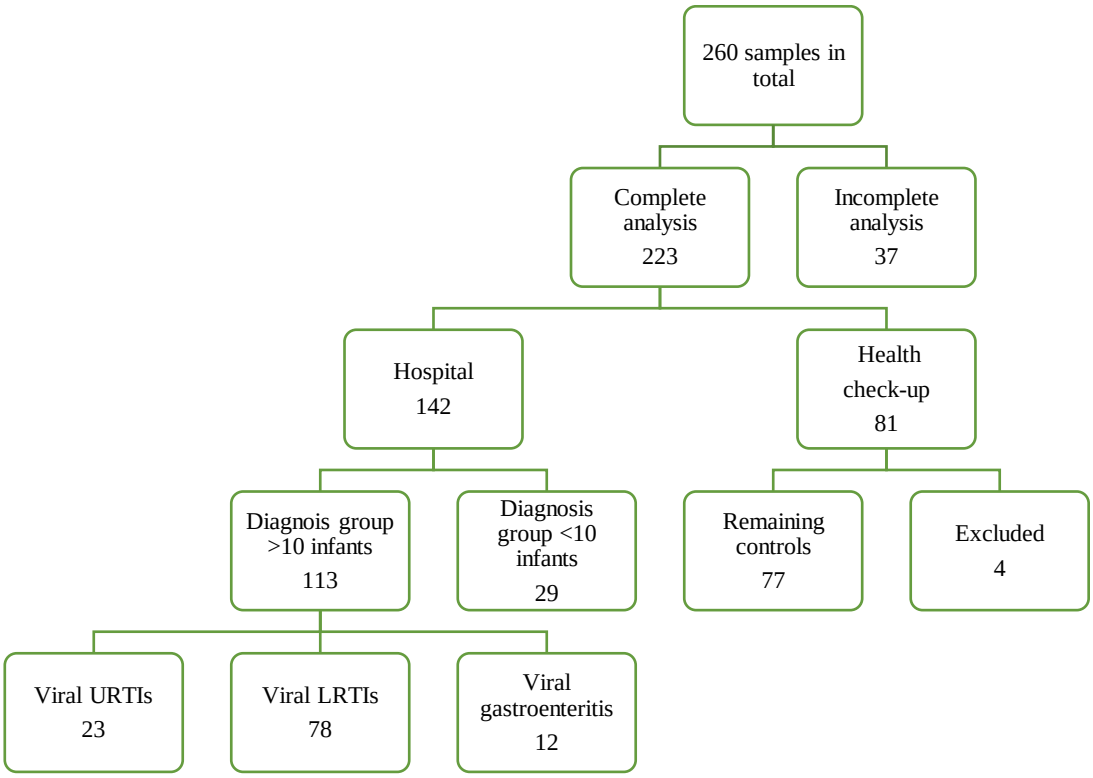

Figure 7: Flow chart showing the process with recruitment/sample collection, sample analysis, separation of cases and controls, verification of exclusion criteria and allocation to diagnosis groups. Modified from Hamrin et al. 2019.

Table 2: Characteristics of healthy controls and hospitalized infants by diagnosis groups.

	Infants n (%) [b]	**Age (days) [c]**	**Male n (%)**	**Days hospitalized [c]**	**CRP (mg/L)**	**Drugs n (%)**
Controls	77	59 (30; 178)	41 (53%)	n/a	n/a	n/a
Viral URTI	23 (16%)	51 (21; 179)	11 (48%)	3 (2; 5)	9 (8; 55)	11 (48%)
Viral LRTI	78 (55%)	86 (44; 152)	49 (63%)	4 (2; 6)	11 (8; 25)	48 (62%)
Gastroenteritis	12 (9%)	270 (126; 325)	8 (67%)	3 (2; 6)	24 (8; 40)	5 (42%)
Bacterial pneumonia	4 (3%)	261 (181, 343)	75%	4 (1; 13)	96 (85; 103)	2
Bronchiolitis + bacterial pneumonia	3 (2%)	32 (11; 34)	67%	7 (4; 7)	51 (16; 68)	1
Pyelonephritis	3 (2%)	45 (7; 66)	67%	3 (2; 4)	51 (18; 60)	3
Others [a]	19 (13%)	92 (29; 151)	63%	2 (1; 6)	11 (8; 57)	7 (37%)

[a] Others (two infants or less with the same diagnosis): febrile seizures, bacterial bronchitis, cutaneous infections, strep A-tonsillitis, reflux, allergic reaction, unspecified feeding difficulties, myocarditis, encephalitis, or chicken-pox.

[b] Percentage of all hospitalized individuals; all other percentages refer to the total number in the specified diagnosis group.

[c] Age, days hospitalized and CRP are expressed as the median and interquartile range.

5.1.3 Adjusting to varying renal function and urine concentration

Our first consideration was to identify the most accurate method to adjust to changes in renal function during infancy as well as for varying degrees of hydration in children admitted with infections.

Renal functions undergo important changes postnatally regarding both glomerular filtration rate (GFR) and tubular functions (secretion and reabsorption). GFR increases by approximately 50% during the first two postnatal weeks and approach adult levels by one year of age. In newborns, the tubular system has a lower capacity for active secretion, while passive tubular reabsorption is increased. Tubular secretion approaches adult levels by 7-12 months of age, while the most pronounced maturation of tubular reabsorption occurs between 1-3 years of age [235].

Body surface can be measured using a subjects' weight and length using specifically developed formulas [236,237]. Using body surface has been shown to correlate well with GFR and has been previously used in measurements of urinary PGE_2 [233,238]. However, it has the disadvantage of being largely unaffected by changes in hydration which consequently affects the concentration of most molecules found in the urine. In other contexts, such as urinary lactate and albumin levels in infants, normalization to urinary creatinine has been used to account for varying urine concentrations [239,240].

In a subset of 13 samples from healthy controls, we had sufficient material for measurement of osmolality, which best reflects the concentration of particles in a solution. In contrast to body surface, we found a strong correlation between urinary osmolality and creatinine (**Figure 8**).

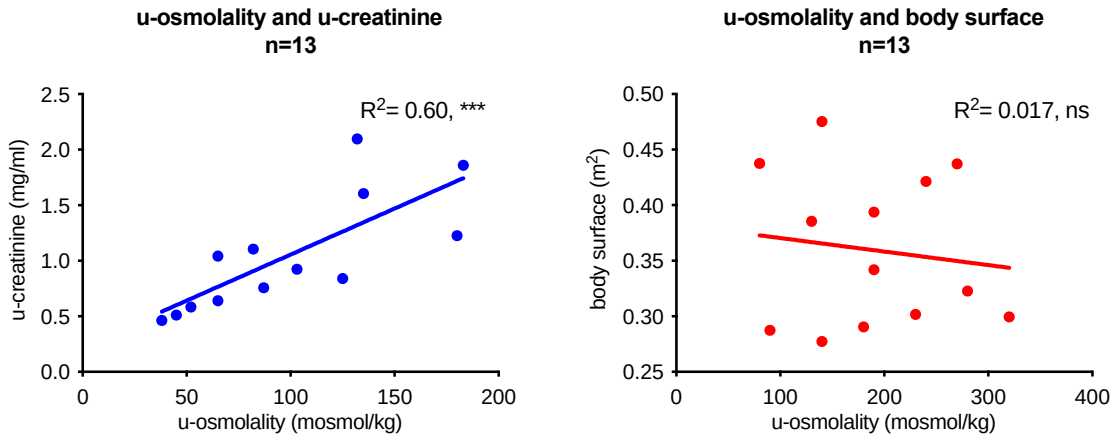

Figure 8: Urinary osmolality compared to urinary creatine (left) and body surface (right). Modified from Hamrin et al. 2019.

From samples taken from all controls, we also found a strong correlation between raw u-tPGEM and u-creatinine, which was not the case for raw u-tPGEM and body surface (**Figure 9**).

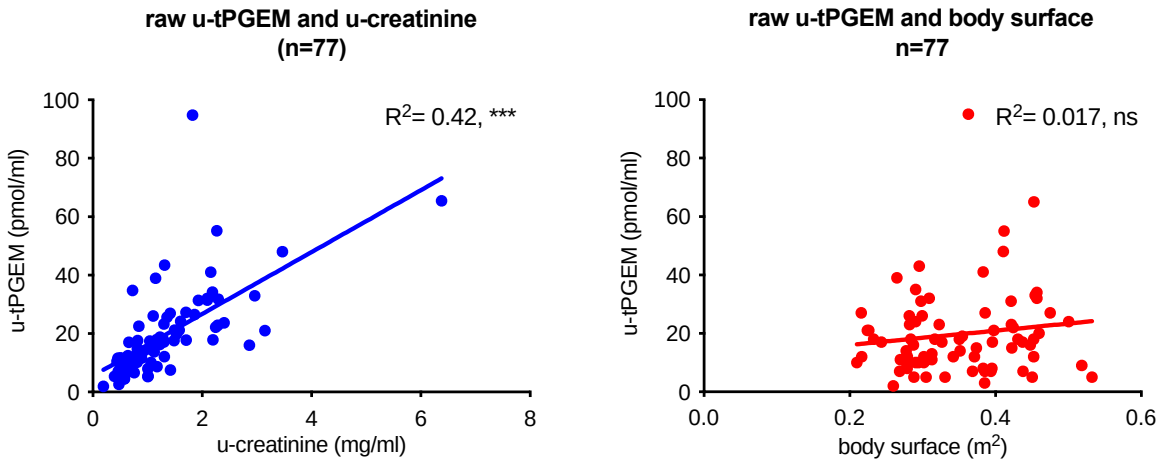

Figure 9: Correlation of raw u-tPGEM with creatinine (left) and body surface (right). Modified from Hamrin et al. 2019.

Based on literature findings, our own results regarding osmolality and the correlation of raw u-tPGEM with u-creatinine, we identified a clear risk of overestimating changes in raw u-tPGEM based on concentration effects when normalizing to body surface. Hence, we concluded that u-creatinine was the best available normalization parameter and if not specified otherwise, u-tPGEM refers to the ratio of raw u-tPGEM/u-creatinine further on.

5.1.4 Age and sex differences

We found no differences of u-tPGEM levels in healthy male and female infants. We found a continuous decline in u-tPGEM levels during the first year of life in healthy infants (**Figure 10**).

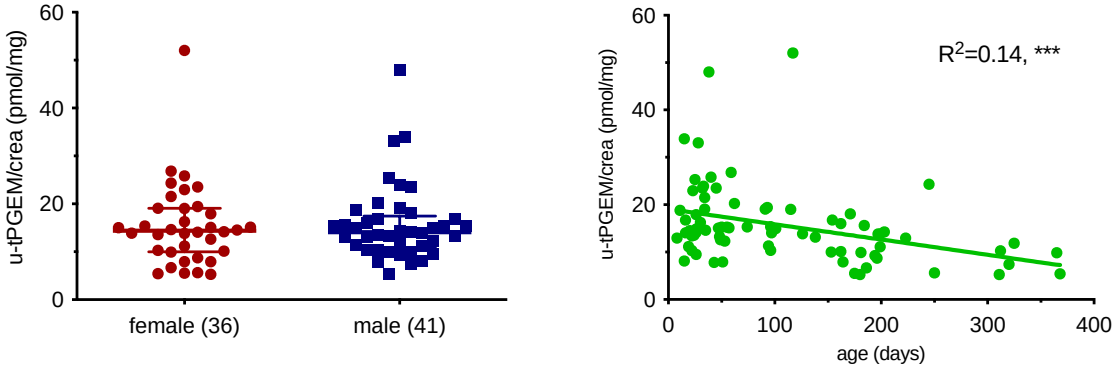

Figure 10: U-tPGEM levels in healthy controls presented for males and females (left) and by age (in days). Modified from Hamrin et al. 2019.

Our findings regarding similar u-tPGEM levels based on sex is in concordance with previous findings regarding urinary PGE_2 levels by Leonhardt et al. However, our findings with respect to subsiding u-tPGEM levels after birth contrast with Leonhardt's findings, which rather saw an increase in PGE_2 levels with increasing age. The difference may be explained by the fact that Leonhardt used the same body surface estimate $1.73m^2$ for all age groups. This fixed body surface estimate poorly reflects that of a growing infant (typical body surface in newborns: $0.25m^2$, at 12 months: $0.56m^2$) and negates the association between body surface and GFR. Consistent with our findings, a study conducted in 1978 by Mitchell et al, showed decreasing levels of PGE2 in plasma in the first eight weeks of life [241].

5.1.5 The effect of paracetamol, NSAIDs and cortisone on u-tPGEM

Documented intake of paracetamol or ibuprofen within 24 hours of sampling or cortisone within 3 days of sampling had occurred in 49% of all hospitalized cases.

Non-steroidal anti-inflammatory drugs (NSAID) elicit their effects by inhibition of cyclooxygenases resulting in diminished PGE_2 levels in tissues and systemically. This mechanism mediates its effects on pyrexia, inflammation and pain. In infants treated with ibuprofen due to patent ductus arteriosus, the treatment significantly reduced urinary PGE_2 levels [242]. Paracetamol has also been shown to function as an indirect inhibitor of predominantly cyxlooxygenase-2 [243–245]. Furthermore, cortisone treatment may reduce PGE_2 levels indirectly through anti-inflammatory effects as well as directly as shown in the human skin and *in vitro* [246,247].

We anticipated a reduction of u-tPGEM levels in infants treated with either of these three classes of drugs. However, in 3 of 4 cases of ibuprofen intake, paracetamol had been taken concomitantly. There were only 3 cases of documented intake of cortisone. We deemed these numbers too small for meaningful analysis and proceeded to investigate the effect of paracetamol intake only. To reduce potential confounding due to type of infection and age, drug effect was studied in 46 age- and diagnosis-matched samples. As presented in **Figure 11**, there were no significant differences.

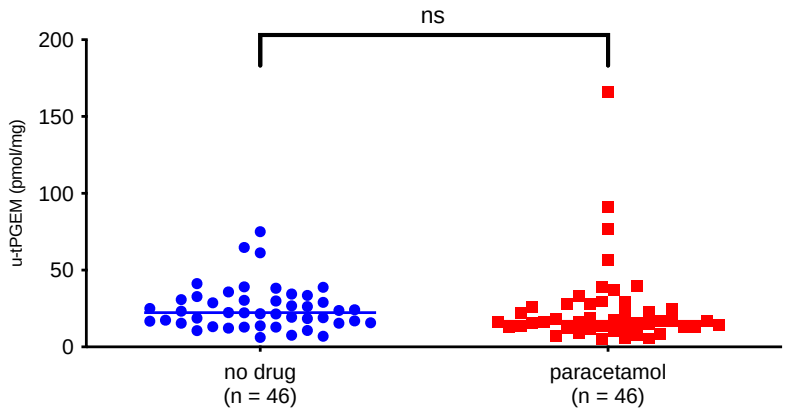

Figure 11: U-tPGEM levels in infants with and without prior treatment with paracetamol matched by age- and diagnosis.

One could argue that the window for prior treatment with any of these drugs was relatively wide, perhaps diluting a possible effect. Based on clinical experience, most infants who have shown a benefit of paracetamol, tend to receive the drug repeatedly. Thus, most infants had received several doses prior to sampling. To thoroughly investigate drug effect on u-tPGEM levels, repeated sampling with defined time-points in relation to drug administration is required. Further on, all samples collected from hospitalized infants were used in subsequent analysis steps, regardless of prior treatment with paracetamol, NSAIDs or cortisone.

5.1.6 U-tPGEM in infected infants

We decided to present results of u-tPGEM levels per diagnosis group with age-matched controls. For each individual in a diagnosis group, an age-matched control was appointed in a process blinded to u-tPGEM results. Because the number of LRTIs (n=78) exceeded the number of controls (n=77), all controls were used in comparison. The age distribution following the matching process is presented in **Figure 12**.

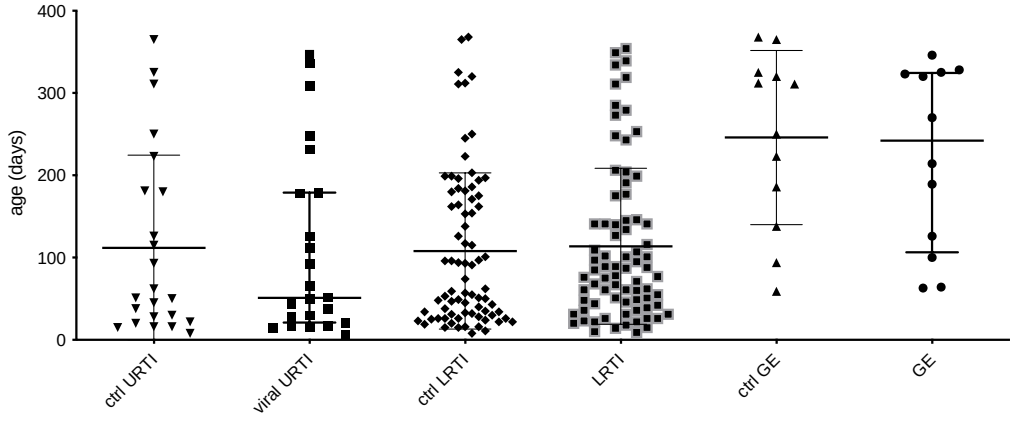

Figure 12: Age distribution in diagnosis groups and their respective control group following age-matching.

The graph shows similar age distributions for GE and LRTIs. Unfortunately, the matching process produced an older control group for URTIs. This creates a risk of overestimating increases of u-tPGEM levels among infants with URTIs as older controls tend to have lower baseline levels of u-tPGEM. Counterintuitive to this, our results showed lower u-tPGEM levels among infants with URTI compared to matched controls and the same levels compared to all controls. In contrast to URTIs, children hospitalized with LRTIs had clearly elevated levels of u-tPGEM as depicted in **Figure 13**.

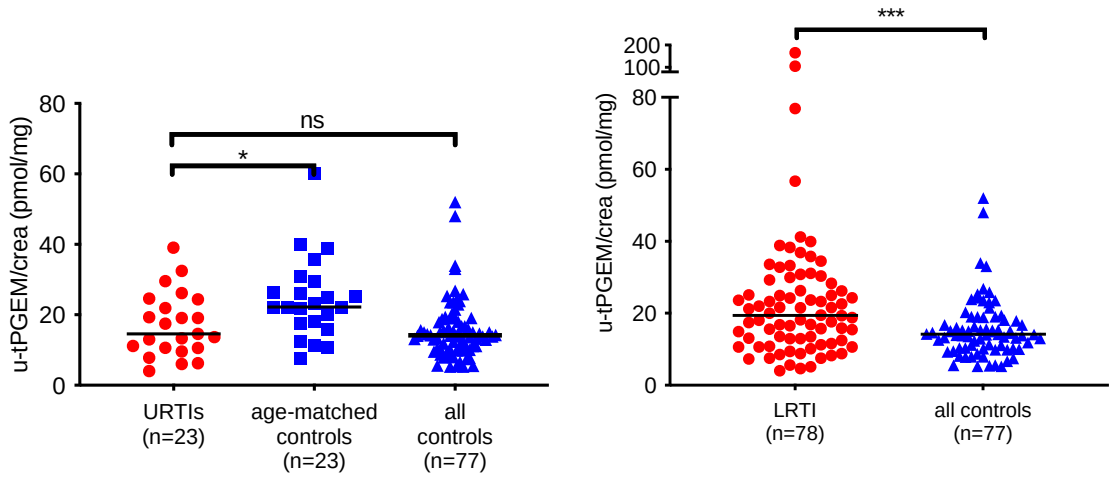

Figure 13: Left: U-tPGEM levels among infants with viral URTIs compared to all and age-matched controls. Right: U-tPGEM levels among infants with LRTIs compared to all controls. Modified from Hamrin et al. 2019.

For children with gastroenteritis, u-tPGEM levels were significantly increased, both compared to all and age-matched controls (**Figure 14**).

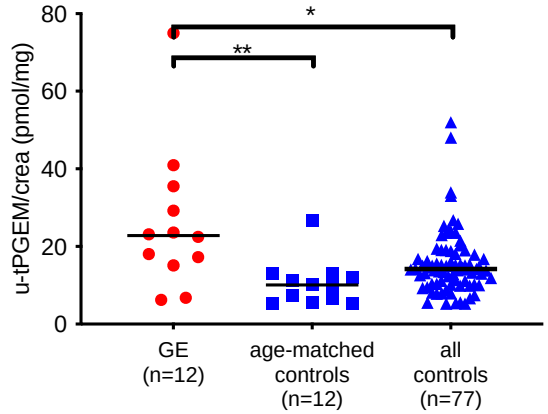

Figure 14: U-tPGEM levels among infants with viral gastroenteritis compared to all and age-matched controls. Modified from Hamrin et al. 2019.

Our data support the notion that an inflammatory response generated to an infection in the lower respiratory tract or in the gastrointestinal tract leads to increased systemic PGE_2 levels measurable as u-tPGEM in the urine. It is beyond the scope of this study to explain the lower u-tPGEM levels found in infants with URTIs. Delineating differences based on viral agent is not possible as only 10/23 URTI-cases had a positive viral finding of which the most common finding was IFV-A. We suggest that the mucosal surface involved in the infection and the subsequent inflammatory response is potentially larger in gastrointestinal and lower respiratory tract compared to the upper respiratory tract resulting in higher u-tPGEM levels. No association between higher u-tPGEM levels and disease severity in terms of length of hospitalization and need for respiratory support was found. Also, the presence of comorbidities had no discernable effect on u-tPGEM levels. To clarify the diagnostic potential of u-tPGEM further, a different study design is required. Ideally this would entail sampling of all infants seeking care at the emergency ward and compare u-tPGEM levels among admitted cases to cases not requiring hospitalization. Furthermore, daily sampling among hospitalized cases could elucidate if u-tPGEM levels correlate with subsequent clinical deterioration.

We found no association between u-tPGEM levels and average temperature in the preceding 24 hours among infants not treated with paracetamol, ibuprofen or cortisone. This weakens the likelihood that systemic PGE_2 levels affect the central nervous system and therefore also the potential link to apneas in infants.

This study adds knowledge regarding baseline levels of u-tPGEM in healthy infants, but also regarding levels associated with infections of the upper and lower respiratory as well as the gastrointestinal tract. Strengths of the study are normalization of the urinary metabolite to creatinine to diminish bias by urine concentration effects as well as age-matched comparisons, which diminish effects resulting from developmental changes. A more standardized sampling procedure would have improved comparability, as systemic PGE_2 levels are expected to vary during the course of an infection. In part, we relied on infants' parents to aid with sampling, who were already involved in caring for their hospitalized child. Given these circumstances, we found it unreasonable to ask for more consistent sampling. Furthermore, full microbial diagnostics would be helpful to delineate differences in u-tPGEM levels relating to site of infection from infectious agents. In a future study, comparing u-tPGEM levels to an age-dependent baseline level rather than age-matched controls could reduce fluctuations resulting from individual variations in controls.

5.2 EPIDEMIOLOGY OF RESPIRATORY VIRUSES IN CHILDREN (STUDIES I, III, V)

5.2.1 Influenza virus epidemics among children in Stockholm, Sweden

The presented results are based on data from two studies (I and III) which in total span over a 18-year period from 1st July 1998 until 30th June 2016 and consistently covering the pediatric population in the northern Stockholm region. A total of 1,050 hospital admissions of children 0-17 years of age with microbiologically verified IFV infections were identified. An additional 297 cases with IFV-diagnosis at discharge were identified for the period 1st July 2016 until 30th June 2019 (study V, in manuscript). Microbial verification of cases from the additional 3 years is ongoing. In recent years there is a concordance of approximately 90% between influenza diagnosis code at discharge and microbial findings, as clinicians tend to diagnose IFV based on PCR results.

5.2.1.1 Influenza epidemics 1998-2019 including all influenza types

For the entire period, this corresponds to an annual incidence of 28.0 per 100,000 (95% C.I. 22.0-34.1) children 0-17 years of age residing within the catchment area. The most severe influenza epidemic in terms of hospital admissions occurred in 2003-04 with an annual incidence of 53.4 per 100,000 driven by the H3N2 Influenza A strain (**Figure 15**).

In a comparable study spanning over 16 years in Finland, Silvennoinen et al. reported an annual incidence of 36 per 100,000 [248]. The somewhat higher incidence in Finland is most likely explained by the lower age-span of included children, which in their case was 0-16 years. For the age group 0-5 years, typically referred to in the literature, we found an average annual incidence of 68.3 per 100,000 (95% C.I. 52.1 – 84.5), which is very much in line with incidences estimated for high-income countries based on a recent meta-analysis [249].

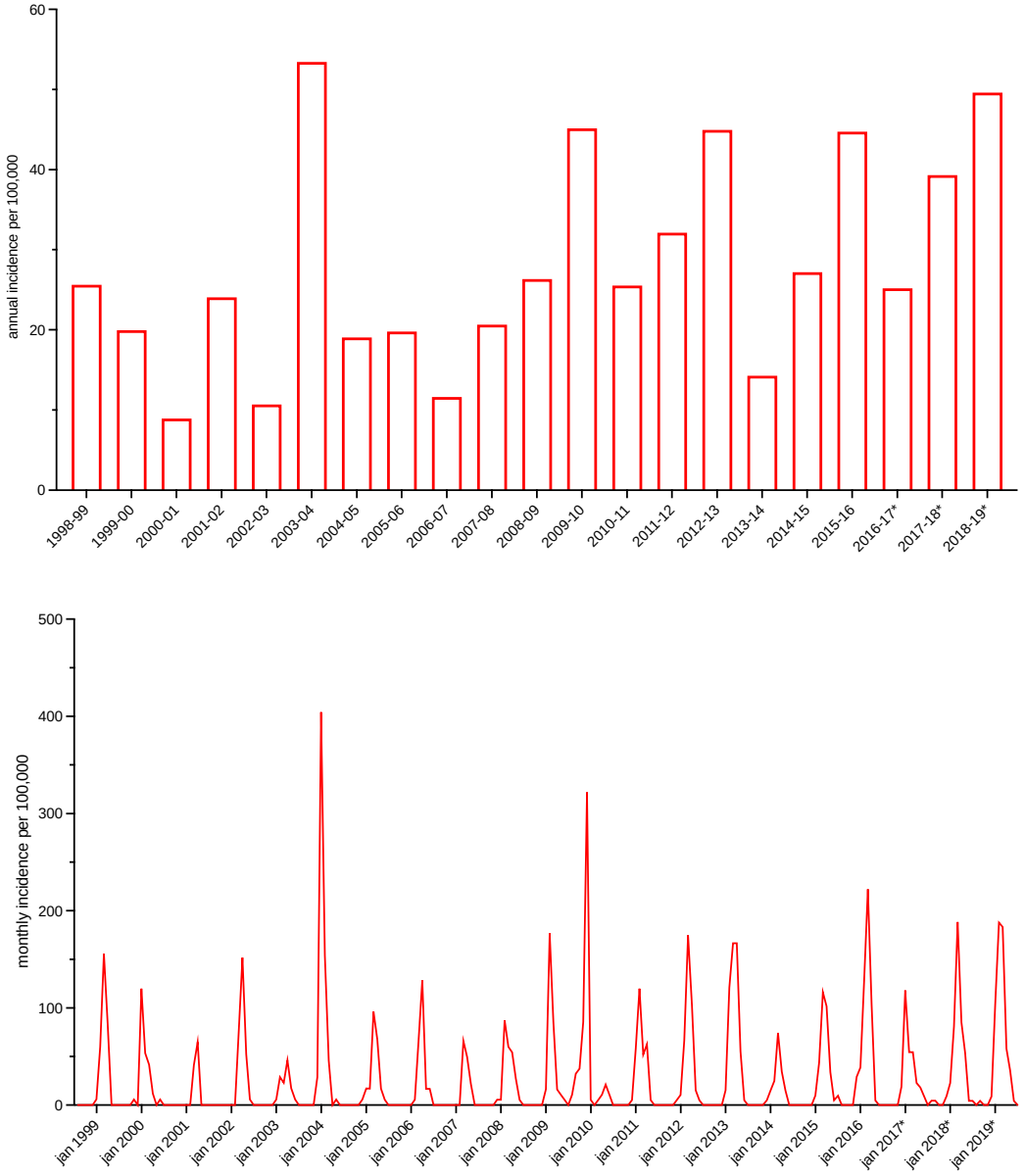

Figure 15: Annual (top) and monthly (bottom) incidence of hospital admissions due to influenza virus in children 0-17 years residing in the Northern Stockholm region 1998-2019. *Cross-verification of IFV diagnosis pending.

Interestingly, a triennial pattern emerged from 2007-08 with ascending incidence rates during a three-year span, followed by a 'reset' at a lower level. This cyclical pattern is less apparent when presenting monthly incidence rates because the influenza epidemics during the 3rd, or 'large' season tend to have a longer duration. In the past 12 years the pattern has been strikingly consistent with four 'cycles' now repeated even though it encompasses the introduction of H1N1pdm09 Influenza A (IFV-A09) in the winter of 2009-10 (**Figure 15**).

A more detailed view of the seasonal epidemics in this cyclical period shows that, apart from the 2009-10 pandemic, 'large' seasons are distinguished by a longer stretch of incidence rates covering the whole winter. 'Small' seasons follow the larger seasons with early peaks

predominantly in December – January. 'Medium' seasons tend to have more distinctive peaks in January – February, more similar to the large seasons. There is also a pattern of early spring drops of large epidemics being followed by earlier peaks of small epidemics and vice versa (**Figure 16**).

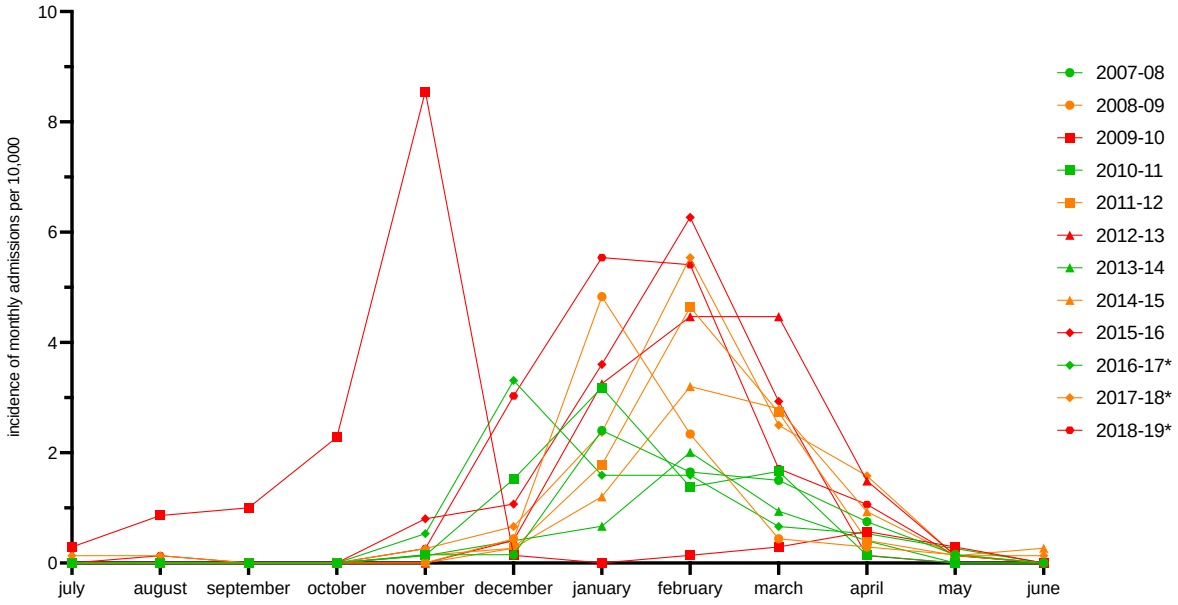

Figure 16: Incidence of monthly influenza admissions in children per season during the period 2007 – 2019. Large influenza epidemics are depicted in red, medium in orange and small epidemics in green.

The observed pattern is highly suggestive of herd immunity as the main driver of influenza epidemics. A periodicity of three seasons per cycle could be explained by a duration of immunity of approximately two years. At this rate, a population will have high immunity in the first season following a large epidemic resulting in a small epidemic. The second season following the large outbreak, immunity is starting to wane, and few susceptible individuals were exposed during the previous winter resulting in a 'medium' epidemic. In the third season following the large epidemic, immunity has waned off from the preceding one, allowing for a new, large outbreak. The waning immunity could be explained by immunological host factors, antigenic drift by the influenza virus or both. It is not evident that immunity to natural infection and vaccination should wane at the same rate. However, it is still worth noting that the effect of Pandemrix® vaccination in 2009-10 was measurable in 2010-11, but no longer in 2011-12 [105].

5.2.1.2 Influenza types found in pediatric hospital admissions 1998-2016

Our results regarding influenza type verified among children are presented in **Figure 17**.

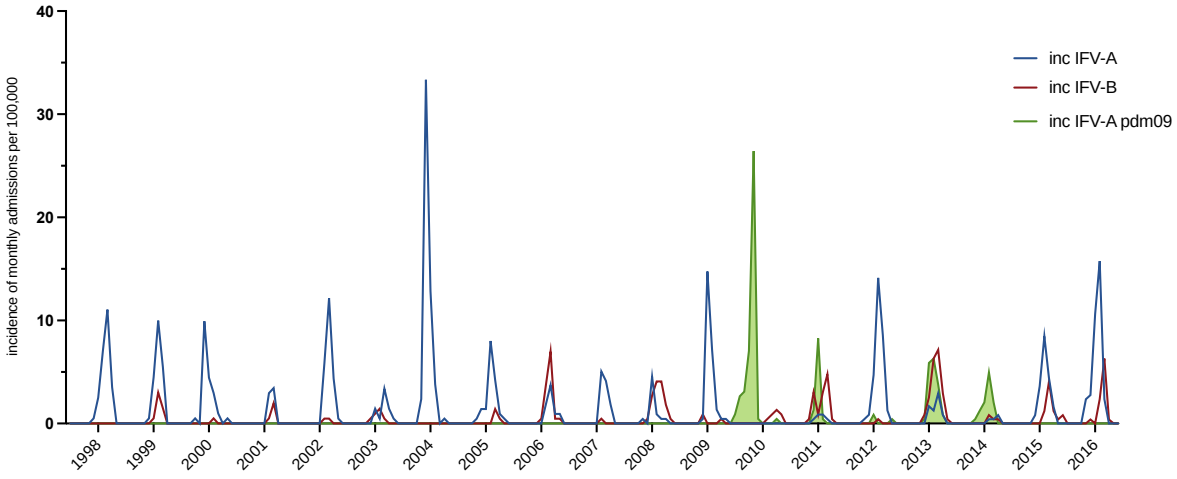

Figure 17: Incidence of hospital admissions in children with seasonal IFV-A (blue), IFV-B (red) and IFV-A pdm09 (green). After 2014, IFV-A pdm09 was no longer specifically investigated.

Associated with the transition from direct IF microscopy to PCR on 1st October 2007 was a marked increase in identification of IFV-B relative to IFV-A ($p < 0.001$, Fisher's exact. **Figure 18**).

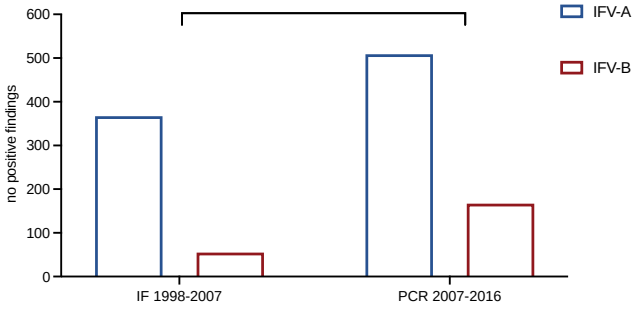

Figure 18: Positive findings of IFV-A and -B based on study period and method.

There are two main explanations for this:

First, this is a true shift in proportions of IFV-A and -B, perhaps due to reduced competition with IFV-A strains. As the aforementioned cyclical pattern with respect to all influenza types suggests, all IFV types appear to compete for the same 'immunological niche'. In this regard, initial IFV vaccinations were trivalent, containing two IFV-A strains but only one IFV-B strain, leaving more 'room' for type B-strains. In addition, the pandemic with IFV-A pdm09 and vaccination programs may have boosted herd immunity towards IFV-A even further [250].

Second, a higher sensitivity for IFV-B in the PCR-assay has led to more positive findings. This is important for IFV-B, which more often infects somewhat older children (se later section), who tend to shed lower viral titers compared to younger children [251,252].

5.2.1.3 Age-dependent attack rates of influenza virus types in children

The previously mentioned age-differences in attack rates for IFV-A, IFV-B and the initial five years with IFV-A pdm09 are depicted in **Figure 19.**

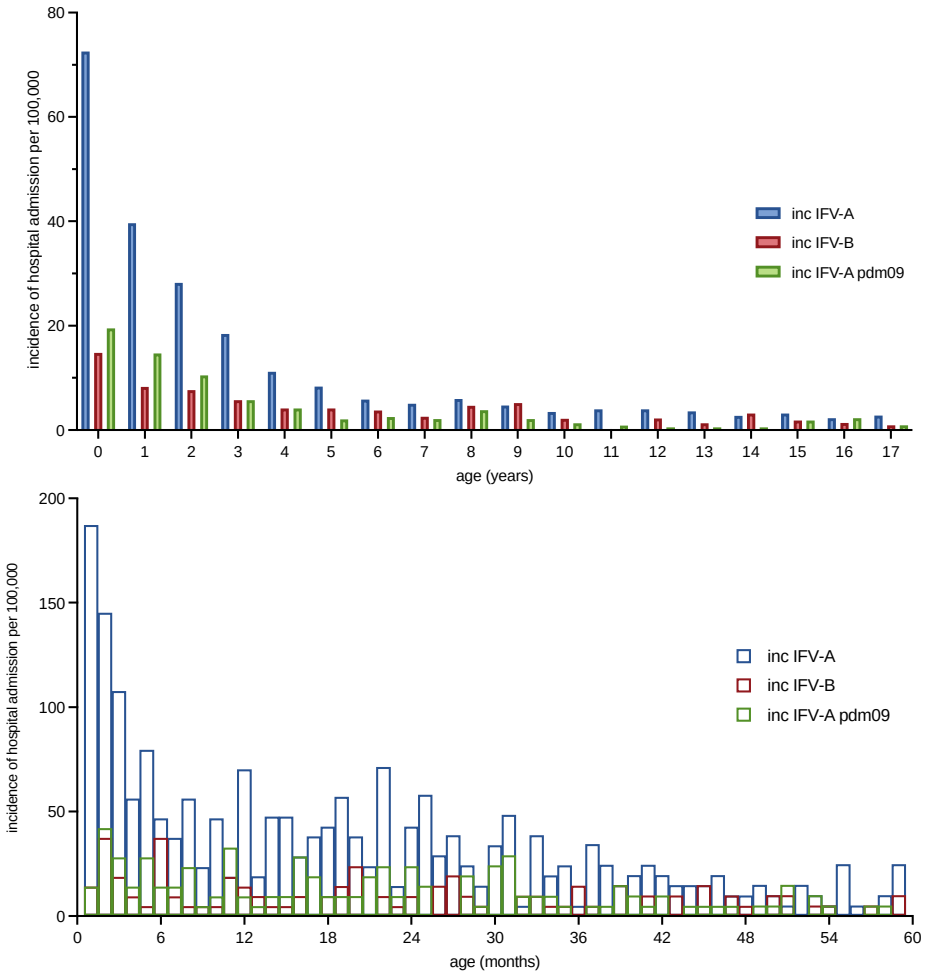

Figure 19: Age-dependent in 0-17 year age group (above) and 0-59 month age group (below), and incidence of hospital admissions due to IFV-A (blue), IFV-B (red and IFV-A pdm09 (green).

The observed pattern of high incidence rates of IFV-A in the first month of life and limited age-dependency of IFV-B has previously been reported in other pediatric influenza studies [248,253]. As will be discussed in later sections, there are only subtle differences in the clinical picture of different IFV-types. If anything, IFV-B tends to be associated with more complications. Therefore, it is likely that the age-differences between influenza types are better explained by age-dependent attack rates in the population.

5.2.2 Respiratory syncytial virus epidemics among children in Stockholm, Sweden

The presented results are based on data contained in studies III and V and covers the same study period (21-years, from 1st July 1998 until 30th June 2019) and population (age 0-17 years, northern Stockholm region) as previously described for results of influenza virus epidemics. Within that frame, a total of 5,253 hospital admissions with microbiologically confirmed RSV infections were included.

5.2.2.1 Results regarding diagnostic method

As previously mentioned, different diagnostic methods were used during the study period. Data relating each method used with age-range in positive findings are presented in **Table 3**. Unfortunately, the type of method used to verify RSV infection was not recorded during the first year after the PCR method was introduced (2007 – 2008).

Table 3: Results regarding diagnostic methods during the study period. Method data not available for entire season 2007 – 2008.

Diagnostic method	Period method in use	Positive findings (% in period)	Median age in months (IQR)
Immunofluorescence	1998 - 2007	1665 (100%)	3.0 (1.4 – 7.6)
Method data not available	2007 - 2008	381 (100%)	3.5 (1.6 – 11.0)
Rapid antigen test	2008 - 2016	1263 (51%)	3.5 (1.5 – 9.5)
Polymerase chain reaction	2008 - 2016	1044 (42%)	6.3 (1.8 – 18.8)
Not documented	2008 - 2016	170 (7%)	20.4 (6.8 – 29.8)
Polymerase chain reaction	2016 - 2019	730 (100%)	3.2 (1.3 – 14.7)

In the period of 2008 – 2016, PCR analysis was only used when initial rapid antigen testing was negative explaining the substantial difference in age between the two methods. Given the high age in positive samples without a documented diagnostic method 2008 – 2016, these were most likely also tested by PCR. A comparison was made between methods used in first-step diagnostics, ie IF 1998-2007, rapid antigen testing 2008-2016 and PCR 2016-2019. The comparison suggests that more recent diagnostic methods identified RSV in older children (**Figure 20**). This should be interpreted with considerable caution given that each test was

used in a different period. The results could become skewed by varying age-specific attack rates of RSV in different time periods, or more likely, changes in sampling practices.

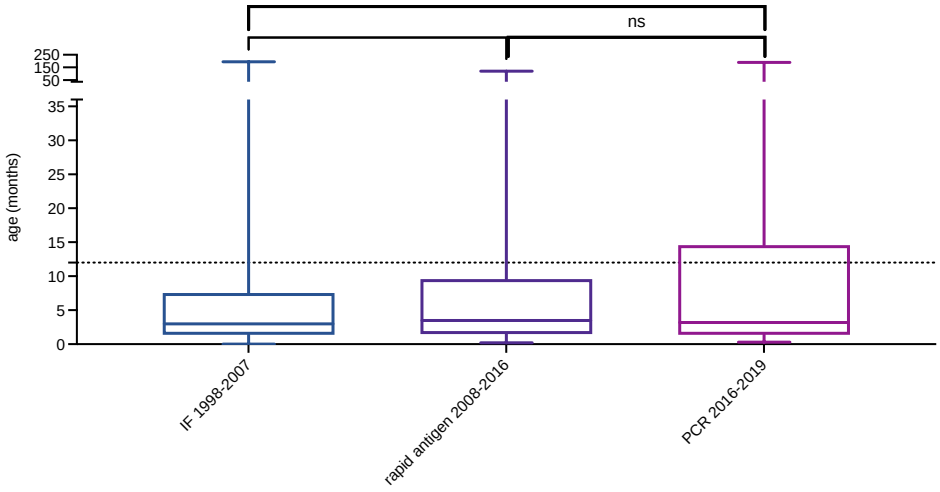

Figure 20: Age differences in different time periods and diagnostic methods in RSV positive children. The dotted line depicts 12 months of age.

5.2.2.2 Age distribution of RSV admissions and siblings

The first encounter with RSV in life is very much determined by the presence of older siblings. First born children typically acquire their first infection as they enter daycare. In Sweden, 40% of children are enrolled at the age of 12 months, by the age of 2 years approximately 90% of children attend daycare according to figures by Swedish National Agency for Education. Infants with older siblings on the other hand, acquire their first infection through transmission from their older sibling early in life (**Figure 21**).

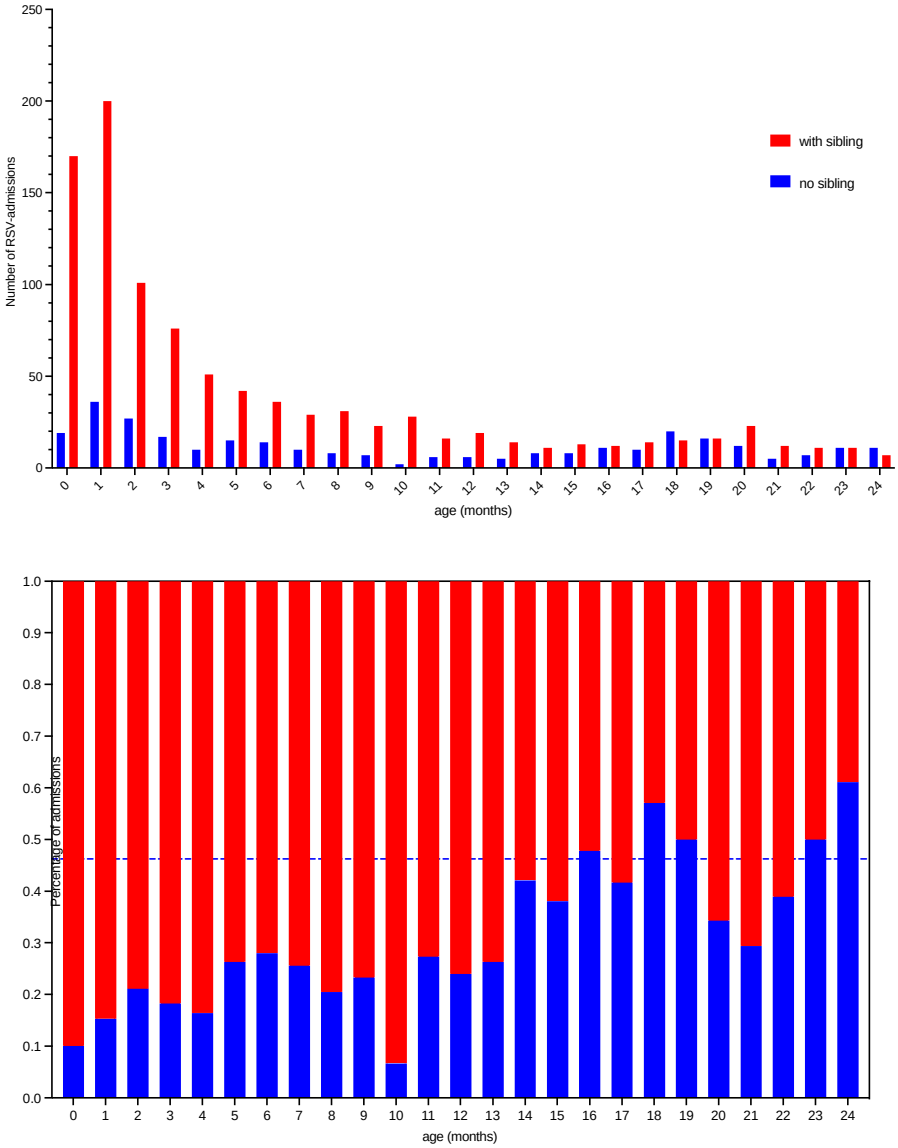

Figure 21: Age of infants admitted with RSV in the northern Stockholm region1998-2019 during the first two years of life. Bars in red represent infants with older siblings, bars in blue infants without siblings. The upper graph depicts number of admissions, the lower graph provides percentage of infants with and without siblings.

Since RSV only circulates during a limited time period, not only the age of a child but also the month of birth is important for its risk to acquire the infection. Based on population data from Statistics Sweden and the Swedish National Board of Health and Welfare, we were able to obtain data regarding monthly number of births as well as the proportion of first-born children from January 2000 until December 2019. Stockholm has considerable variations in birth rates over the year (**Figure 22**), but the percentage of first-born infants has remained fairly stable around 46% (data not shown).

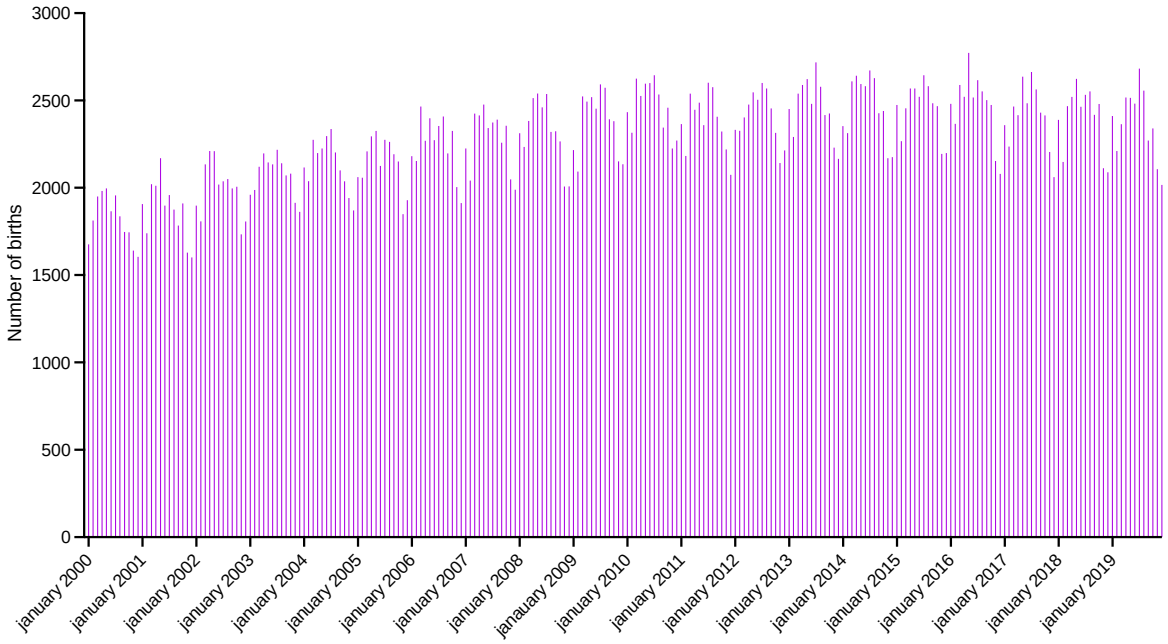

Figure 22: Number of births per month in the Stockholm region January 2000- December 2019.

From this data, we obtained the average number of births based on calendar month for the study period among first-born infants and infants with siblings. We used these figures to calculate the incidence of hospital admission based on age and month of birth per child residing in the Stockholm region. The results of the combined data are presented as heatmaps in **Figure 23** for infants with and without siblings. The figure illustrates the strong affect the combination of age, month of birth and presence of an older sibling has on the risk to require admission due to an RSV infection.

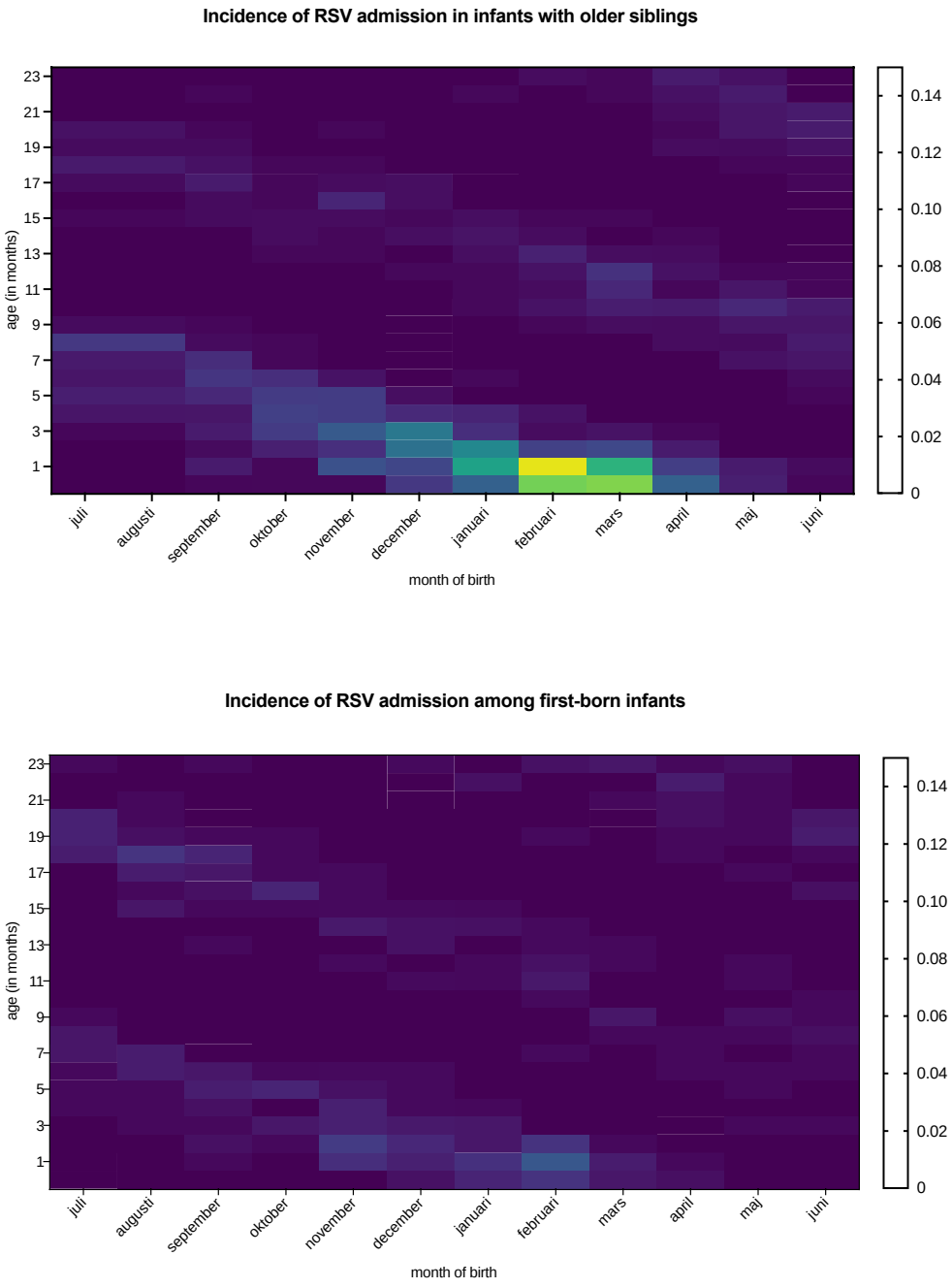

Figure 23: Incidence of hospital admissions per child residing in Stockholm with RSV by month of age (0-23 months) and month of birth.

5.2.2.3 RSV epidemics in Stockholm 1998 – 2019 in children <1 year of age

To reduce the aforementioned variations in epidemic pattern related to viral detection methodology, we included only hospitalized cases <1 year of age (3,907, or 74% of all cases). Previous sections have described the delayed biennial pattern observed in several temporal regions [114,254]. Delayed biennial refers to epidemic seasons with one large, early winter epidemic followed by a smaller epidemic with a delayed onset. Marked seasonal variations in temperature and humidity provide conditions for large RSV outbreaks in winters

and nearly no detectable virus transmissions in the warmer months [115,188]. Mathematical models suggest that a prerequisite for this pattern is a duration of immunity for 7.5-10 months and birth rates 0.0125 – 0.0137 [188,190,255]. The duration of immunity is important because it contributes to a delayed start and lower incidence rates of an epidemic following a big epidemic in the preceding year. The small epidemic in turn leads to lower herd immunity, thus providing conditions for a larger outbreak the following winter. Birth rates are important because of the high attack rates of RSV in young children, with 90% of children having experienced at least one RSV infection by the age of two (**Figure 21 and 23**)[256]. In this respect, birth rates have a substantial effect on the pool of susceptible hosts in an epidemic. If birth rates are low, the number of newborns will be too small to substantially affect the number of susceptible individuals in seasonal RSV epidemics, resulting in equally small, annual epidemics. Very high birth rates on the other hand, will renew the pool of susceptible individuals thereby diminishing the effect of residual herd immunity from the previous season. Thus, very high birth rates result in large, annual epidemics.

The delayed biennial pattern has been observed in the Stockholm region in studies conducted 1984 -1993 and 1987 – 1998 [8,257]. During the herein studied 21-years, this pattern has remained similar, but more or less pronounced. However, following the influenza pandemic in 2009, we observed two consecutive large RSV outbreaks. These were followed by a restart of the delayed biennial pattern, but with an inverse rhythm: Large, early epidemics now occurred on odd-numbered years prior to 2009-10. From 2011-2012, odd numbered years coincided with smaller and later peaks (**Figure 24**).

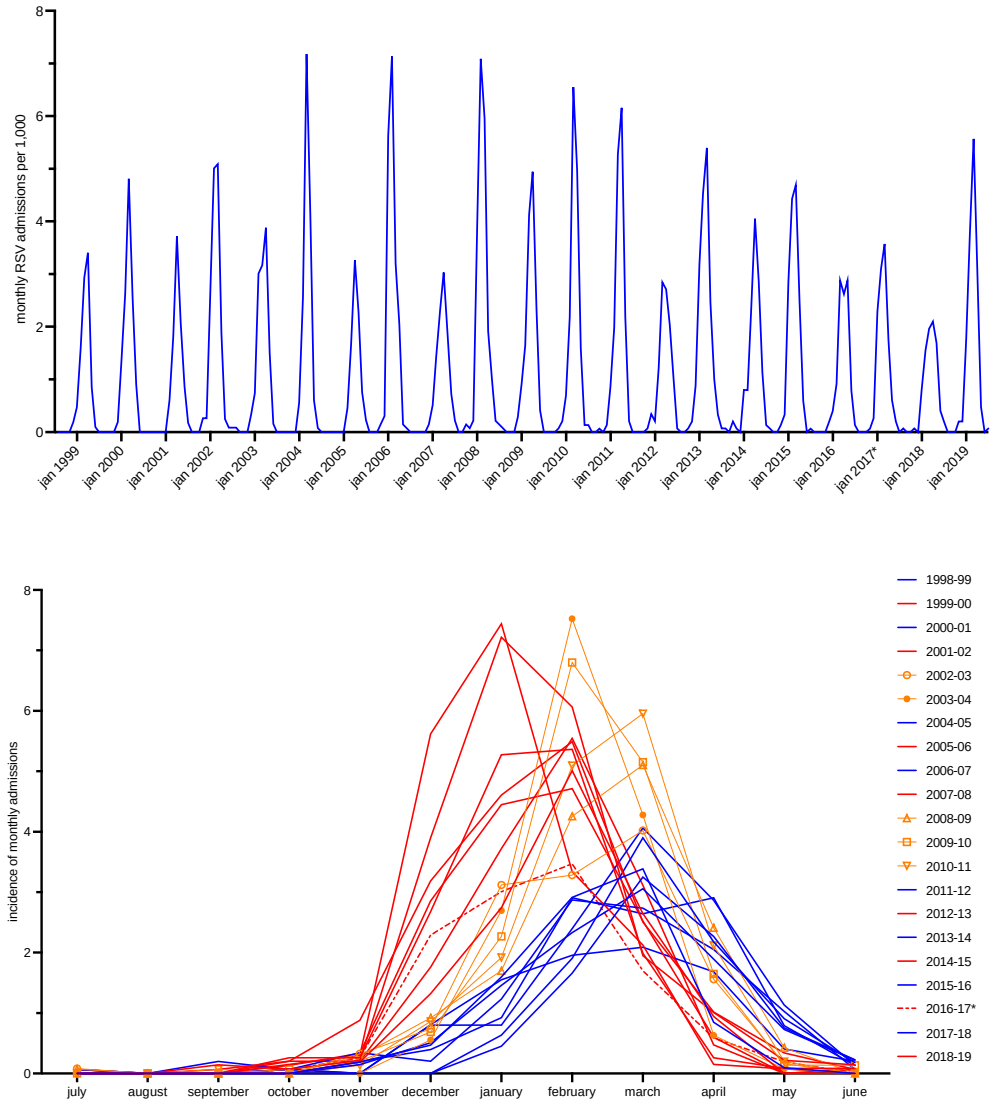

Figure 24: Incidence of hospital admissions due to RSV among children <1 year in the northern Stockholm region 1998 – 2019. The top graph shows monthly incidences spread over the entire study period. The bottom graph shows the monthly distribution of incidences for each season. We identified three types of epidemics relating to timing and size of the peak: early/large in red, late/small in blue, intermediate in orange. * In 2016-17 the children's hospital had a reduced capacity for admissions due to relocation.

As presented in **Figure 24**, most RSV epidemics in the study period are easily allocated into early/large or late/small. Early/large epidemics tend to start in November with a marked increase of hospital admissions and peak by January-February. Late/small epidemics on the other hand tend to have a much more subdued inclination in admissions starting in December – January that peak by March. However, during five 'irregular' seasons, RSV epidemics fit neither pattern as they presented with a steep increase of admissions in December – January. We attempted to explain these 'irregular' seasons, particularly the ones in 2009-2010 and 2010-2011 that resulted in the inversion of the biennial pattern based on three main hypotheses: 1. Temporary changes in birth rates affected herd immunity. 2. Changes in

meteorological conditions affected RSV transmissibility. 3. Viral interference due to large IFV epidemics affected RSV transmissibility.

5.2.2.4 Birth rates in Stockholm during the study period

There was a substantial increase in birth rates during the study period with rates around 0.01 in 1998, steadily increasing to a peak around 0.0144 in 2009. There was a gradual decline in birth rates to 0.012 during the last half of the study (**Figure 25**).

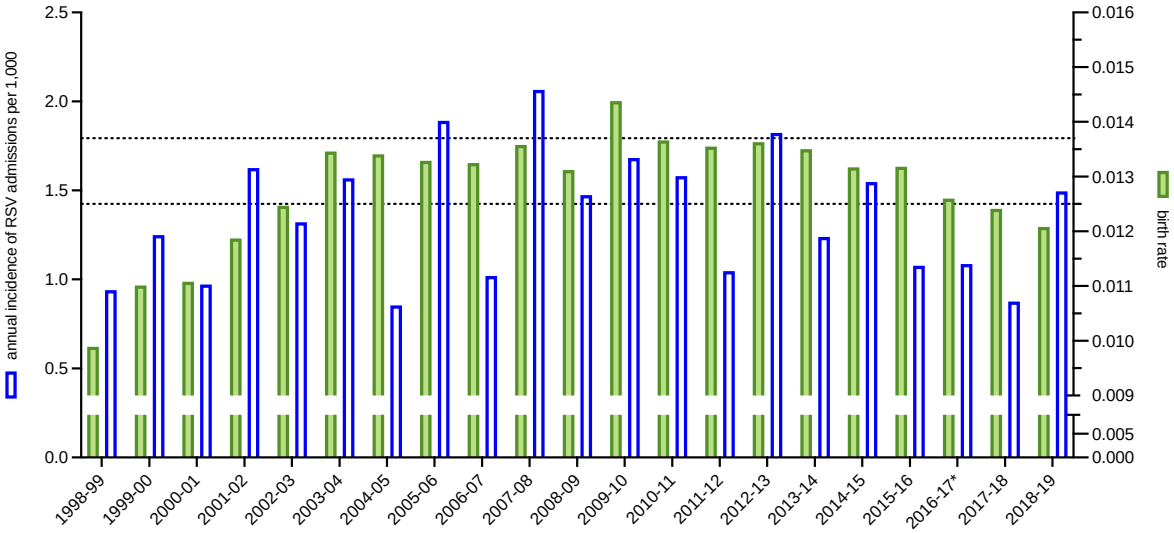

Figure 25: Annual birth rates in Stockholm (in green, right y-axis) and annual incidence of RSV admissions in children <1 year of age per 1,000 (in blue, left y-axis). Dotted lines set at birth rates of 0.0125 and 0.0137 respectively. *Admission rates possibly reduced because of limited numbers of beds due to hospital relocation.

It is interesting to note that the most pronounced oscillations between small and large epidemics coincide with periods were birth rates remained within 0.0125-0.0137. This was the range predicted by mathematical models in populations with delayed biennial RSV epidemics. With this in mind, the 10% increase and high total birth rates of 2009 could have tilted the populations immunological balance into high, annual peaks as previously mentioned. However, in the season of 2009-10 a large, early epidemic was “due” based on the previous smaller epidemic. It appears counterintuitive that higher birth rates would delay the start of the epidemic.

5.2.2.5 Temperature changes in Stockholm during the study period

As previously mentioned, outdoor temperature may affect virus transmission directly or indirectly by affecting humidity and perhaps also indoor crowding. We studied possible variations in winter temperatures based on the Swedish Meteorological and Hydrological Institute’s figures on average monthly temperatures in Stockholm 1998-2019 (**Figure 26**).

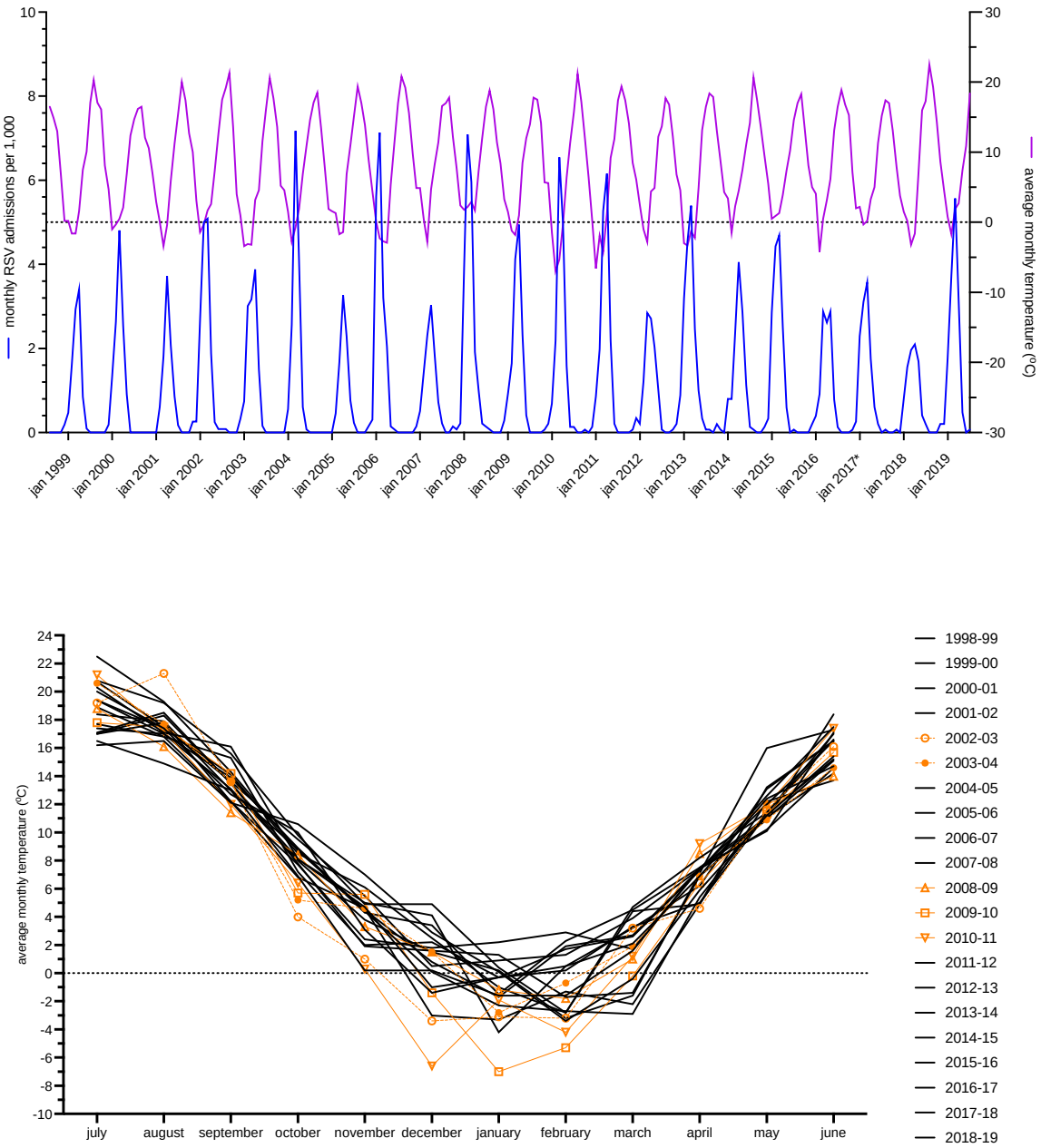

Figure 26: Average monthly temperatures during the study period. The top graph depicts monthly temperature averages (in purple, right Y-axis) with monthly incidences of RSV admissions per 1,000 (in blue, left y-axis). The bottom graph compares annual variations in temperatures with curves for irregular RSV seasons in orange and regular in black.

Figure 26 shows that both winter of 2009-2010 and 2010-2011 were exceptionally cold compared to the rest of the study period. Assuming that low temperatures increase virus transmissibility, the cold winter in 2010-11 could have led to higher incidence rates and an earlier start of the epidemic than expected. However, the contrary is true for 2009-10, which had a delayed start. For the entire study period, the evidence for a direct impact of temperature on incidence rates appears unclear. For example, one of the largest RSV

epidemics during the study period in 2005-2006 coincided with the warmest winter of the entire study period. A strong direct impact of temperature on virus transmissibility is also hard to reconcile with the prospect of delayed biennial epidemics. We conclude that climate, rather than temporary temperature variations define RSV epidemics in a more general manner.

5.2.2.6 Evidence for viral interference between IFV and RSV in Stockholm

Our inquiry regarding suspected viral interference between IFV-A pdm09 and RVs in the summer and fall of 2009 in **study I** highlighted the importance of using extended study periods when investigating viral interference from an epidemiological perspective [258]. As previously mentioned, virus to virus interactions can occur on a host tissue level, immunological level as well as on a behavioral level. An epidemiological study cannot distinguish between these levels and therefore the herein used term of viral interference encompasses all these levels. Again, to account for possible variations in virus detection and consequently age-group captured by IF and PCR methods, we only compared admissions from infants <12 months with RSV and <5 years with any type of IFV. To compensate for the growing population, incidence rates were used rather than absolute numbers of hospital admissions (**Figure 27**).

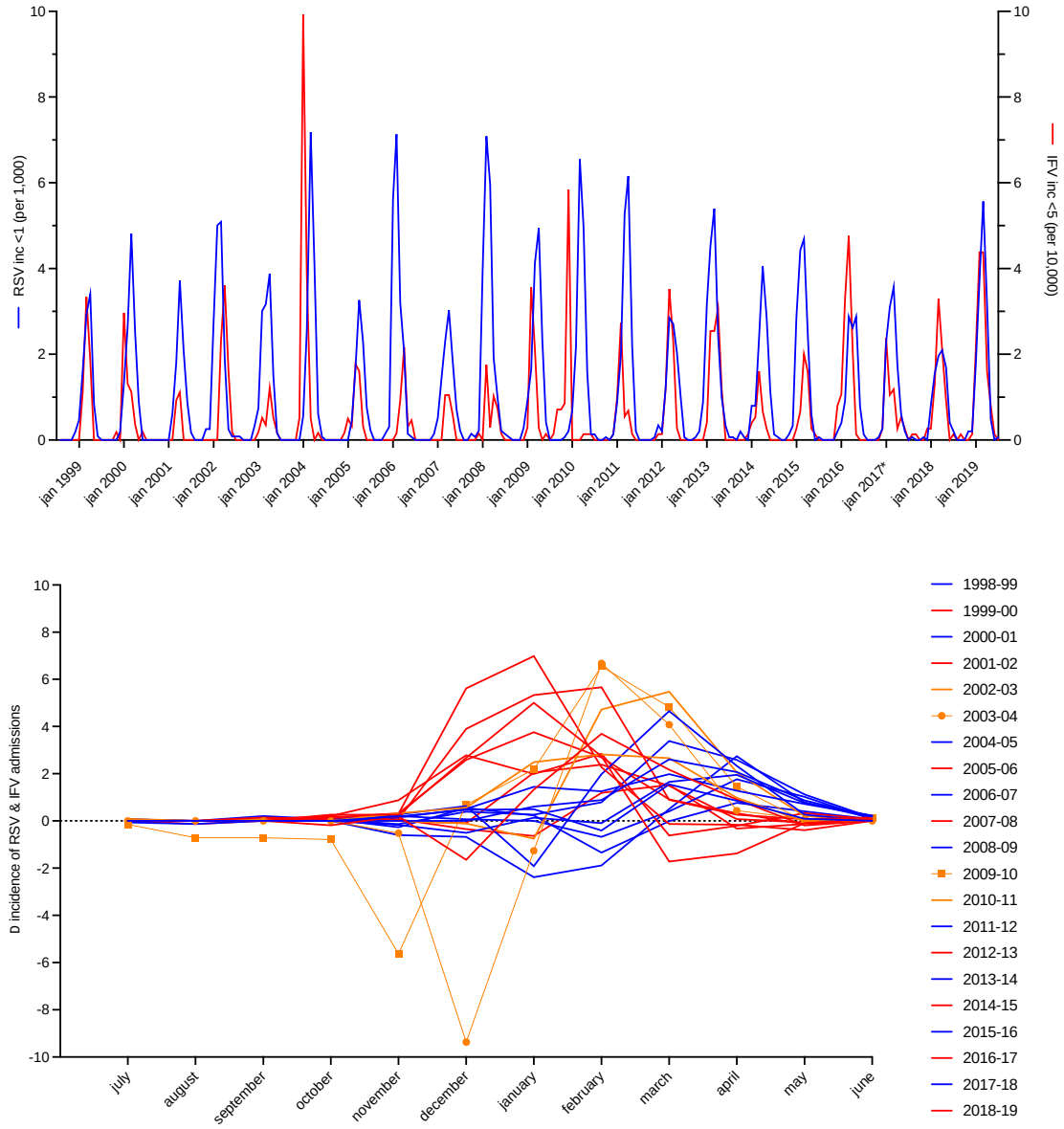

Figure 27: Top graph: Incidence of monthly admissions for RSV per 1,000 (in blue, left y-axis) and for IFV per 10,000 (in red, right y-axis) in the northern Stockholm region 1998 – 2019. Bottom graph: difference in RSV incidence (per 1,000) and IFV incidence (per 10,000) per season. Large/early RSV seasons are depicted in red, late/small seasons in blue and irregular seasons in orange.

In most seasons, IFV and RSV epidemics coincided with the notable exceptions of 2003 – 2004 and 2009 – 2010, which were also the two largest epidemics in the studied period. On both occasions an early, large RSV epidemic was "due" but commenced later in the winter with an irregular pattern. Strengthening the case for viral interference, the shifts in RSV-epidemics following the influenza pandemic of 2009 has been observed around the globe [129,213,259,260]. However, in all these reports, the changes to RSV epidemics were constrained to same season. To our knowledge, no other country has observed the sustained inversion of delayed biennial epidemics. In part, this could be explained by the fact that in many populations, RSV epidemics have more "robust" annual pattern. From a herd immunity

perspective, it appears counterintuitive that the delayed start of the RSV epidemic in 2009 – 2010 should provide conditions for the late/small epidemic that was "due" in 2010 – 2010 to commence earlier with a relatively high peak.

5.2.2.7 Conclusions regarding disruption of RSV epidemic pattern

The disruption of RSV epidemic pattern of 2009 – 2010 and 2010 – 2011 was associated with changes to all hypothesized causes: a 10% increase in birth rates, two exceptionally cold winters and an influenza pandemic caused by a new IFV-A strain. This makes the task of attributing and excluding causal factors near impossible, and it could well be argued that a 'perfect storm' affecting several epidemic drivers at once is required for the observed pattern change to occur. None the less, based on observations from the entire study period we would suggest a hierarchy of causal factors:

1. Birth rates. As we have shown, RSV epidemics are driven by young children. A sudden increase in birth rates will decrease herd immunity, especially in the following year, as this birth cohort enters daycare. We believe this had the strongest impact in 2010 – 2011.

2. Viral interference. Shifts in RSV-peaks were observed on two occasions in our study period and has been reported in other settings. We suggest that the interfering effect subsides with epidemic/pandemic of the competing virus. In the case of the influenza outbreaks, they were relatively brief compared to the SARS-CoV-2 pandemic which appears to have subdued RSV- and influenza almost entirely for 18 months. Perhaps as a consequence, we are now experiencing a substantial rise in RSV-cases in children, most likely due to low herd immunity.

3. Temperature. We believe this to be the least likely contributor to a substantial change in an otherwise robust epidemic pattern. Both from a theoretical point of view and with respect to the delay in 2009 – 2010, exceptionally cold winters appear to be the least plausible cause.

5.2.2.8 Strengths and limitations

Epidemic studies covering more than two decades of microbiologically confirmed RSV- and influenza cases in children in a defined population are rare. We have sought to explain changes in epidemic patterns from different perspectives with meteorologic and population data relevant to the study base and study period. By limiting the age group studied, we have tried to address possible bias due to increased sensitivity with new vial diagnostic assays. An important limitation to our study is that recruitment is entirely dependent on hospital admissions, which represents the 'tip of the iceberg' when it comes to viral respiratory infections. For more accurate description of viral epidemics, representative viral testing of outpatient cohorts and healthy controls should be conducted. Given the resources required for such a study, the 'tip of the iceberg' approach is acceptable. Another caveat is that the rate of

hospital admission may change over time. In our setting, pediatric hospital beds have declined in total numbers and even more so in relation to population growth. Moreover, the fact that PICU admission for the < 1 month old infants appear to be lower during the peak of RSV epidemics, i.e. January – February, suggest that we may be underestimating incidence rates (**Figure 28a+b**).

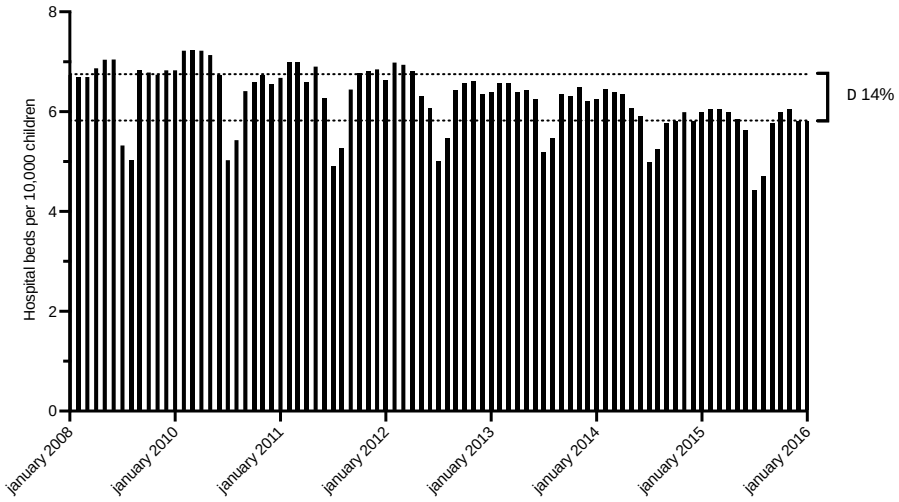

Figure 28a: Number of hospital beds per 10,000 children residing in the hospital's catchment area 2008 – 2016.

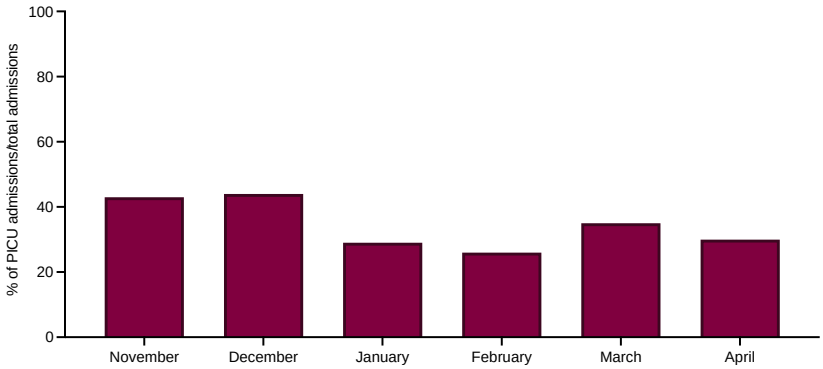

Figure 28b: Percentage of PICU-admissions among <1 month old infants with RSV, 2008 – 2016.

However, based on our experience, a shortage in hospital beds during peaks of viral epidemics tend to affect children with other diseases to a larger extent due to the immediate nature of respiratory distress. As an example, elective procedures are often postponed in situations of shortage. These adaptations improve hospital bed accessibility for children with respiratory infections but also widens the scope of impact related to viral epidemics.

5.3 CLINICAL PRESENTATION OF VIRAL INFECTIONS IN CHILDREN (STUDIES III AND IV)

The current knowledge regarding risk factors and complications in viral respiratory infections in general, have been previously reviewed. This chapter will focus on presenting our findings and discuss its implications. To meet the aim of presenting a full picture of the clinical presentation of influenza in children, no age cut-off was used as in the previous epidemiological analyses. This implies that older children hospitalized with influenza are more likely to be identified in the 'PCR-era' compared to the 'IF-era'.

5.3.1 Risk factors among children hospitalized with influenza virus

The results presented here are based on 888 children hospitalized with confirmed influenza from 1st July 1998 until 30th June 2013 at Astrid Lindgren Children's Hospital.

5.3.1.1 Risk factors among children hospitalized with influenza viruses

Table 4: Risk factors in children admitted with IFV-A, IFV-A pdm09 and IFV-B. Significance of age differences were tested with ANOVA.

	Influenza A n = 553	Influenza A pdm09 n = 160	Influenza B n = 175
Median age in years (IQR)	1.9 (0.5 – 4.5) **	2.3 (0.9 – 6.5) **	4.0 (1.5 – 8.8) **
No preexisting condition	349 (63%)	94 (59%)	95 (54%)
Any preexisting condition	204 (37%)	59 (37%)	80 (46%)
Asthma	50 (9%)	18 (11%)	18 (10%)
Chronic heart disease	10 (2%)	2 (1%)	5 (3%)
Chronic lung disease	25 (5%)	6 (4%)	9 (5%)
Prematurity	21 (4%)	9 (6%)	3 (2%)
Neuromuscular disease	80 (14%)	19 (12%)	30 (17%)
Immunodeficiency	20 (4%)	10 (6%)	10 (6%)
Other	11 (2%)	4 (3%)	7 (4%)

Our data reveals no significant differences in the distribution of risk factors depending on influenza type. The trend of a higher proportion of children with risk factors in the IFV-B group may be explained by the difference in age-dependent attack rates of IFV-A and IFV-B as young age is a risk factor in itself. An older child with risk factors is more likely to require hospitalization for an IFV infection than a healthy child of the same age. To some extent, a risk factor may be evaluated by how late in childhood influenza admission still occurs. This is not as applicable for conditions arising in young children, i.e. prematurity, or conditions arising later in life, i.e. oncologic diseases. More important indicators of the importance of a risk factor are length of hospital admission and admission to the PICU (**Table 5**). 56% (vs 51% in the population) of all admissions were male, which is in line with previously mentioned observations of overrepresentations of males in association with infectious diseases. There were no differences in terms of age, length of admission and PICU admission between males and females (data not shown).

Table 5: Age, length of stay and PICU admission in hospitalized children with IFV of any type. Statistical comparisons were made with healthy children as the reference group. Continuous variables were tested with ANOVA, categorical variables with Fisher's exact.

	Median age in years (IQR)	Median length of stay in days (IQR)	PICU admission (%)
No preexisting condition	1.6 (0.4 – 4.3)	2.0 (1.0 – 3.0)	34 (6%)
Any preexisting condition	3.1 (1.4 – 7.3) ***	3.0 (2.0 – 6.0) ***	66 (19%) ***
Asthma	2.5 (1.8 – 5.3)	2.0 (1.0 – 3.0)	2 (2%)
Chronic heart disease	0.9 (0.6 – 2.9)	2.0 (1.3 – 4.0)	2 (12%)
Chronic lung disease	1.5 (0.5 – 3.4)	2.0 (4.0 – 7.0)	15 (38%) ***
Prematurity	0.4 (0.2 – 1.0)	4.0 (2.0 – 9.0)	5 (16%)
Neuromuscular disease	4.9 (2.6 – 9.1) ***	4.0 (2.0 – 8.0)	37 (29%) ***
Immunodeficiency	8.0 (4.4 – 15.9) ***	3.0 (2.0 – 5.0)	3 (8%)
Other	3.4 (1.5 – 9.4) *	2.0 (1.0 – 5.0)	4 (18%)

Unsurprisingly, having any precondition is associated with longer hospital admission and a substantially higher risk of requiring admission to the PICU. The relatively mild courses among children with asthma, chronic heart disease and prematurity may be a reflection of successful vaccination programs for risk groups, including pregnant women. Children with chronic lung diseases may require respiratory support even when not infected, hence their PICU admission rate may be overestimated. The relative short length of stay in this risk group strengthens this notion. Based on our findings, children with neuromuscular diseases are at highest risk for severe disease as reflected in both longer duration of admission and the elevated risk to require PICU admission. This has also been reported by others and underscores the importance of good vaccination coverage for this group of children [86,101]. The short length of admission and low risk for PICU admission among children with immunodeficiencies including malignancies and those with asthma are also in line with previous findings[4,62,90,93].

5.3.2 Complications among children hospitalized with influenza viruses

Complications were investigated using the same methodology as previously described. The data also encompasses 888 pediatric hospitalizations spanning over the period 1998 – 2013. In cases where children had more than one complication, both were counted separately.

Table 6: Complications/manifestations per influenza type among hospitalized children in total numbers and as a percentage of children with the same influenza type. Statistical difference of myositis among IFV-B calculated in comparison to IFV-A with Fisher's exact.

	Influenza A n = 553	Influenza A pdm09 n = 160	Influenza B n = 175
No complication	299 (54%)	93 (58%)	77 (44%)
Obstructive bronchitis	50 (9%)	16 (10%)	20 (11%)
Croup/tracheitis	18 (3%)	3 (2%)	12 (7%)
Pneumonia / Empyema	70 (13%)	20 (13%)	21 (12%)
Seizures	70 (13%)	12 (8%)	19 (11%)
Encephalitis	24 (4%)	1 (1%)	8 (5%)
Myositis	2 (<1%)	2 (1%)	8 (5%) ***
PICU	57 (10%)	17 (11%)	26 (15%) ns
Deaths	1 (<1%)	2 (1%)	1 (1%)
Other	20 (4%)	10 (6%)	11 (6%)

The only significant difference in complications related to IFV type, was myositis which was more common in IFV-B. This is well in line with the literature, where IFV-B has been attributed to 70% of microbiologically verified influenza associated myositis [53]. The higher percentage of PICU admission was among children hospitalized with IFV-B was not statistically significant. Our results, including prevalence of neurologic complications is in line with other reports [87,100].

5.3.3 Clinical aspects among children hospitalized with respiratory syncytial virus

Presented results are again based on hospitalized children residing in the hospital's catchment area. The study period covers 1st July 2008 until 30th June 2016. As previously mentioned, and described in detail in **Table 3**, the virological method for RSV identification was the rapid antigen test at admission (50% of positive findings). Negative cases were subsequently tested with PCR. A total of 2351 cases with a clinical and microbial diagnosis of RSV were identified. In 7% the method for microbial testing was not documented. 58% of all cases were male. In contrast to our publication of study IV [261], recurrent wheezing and asthma was included as a risk factor in this analysis.

Table 7: Risk factors among hospitalized children with RSV in Stockholm 2008 – 2016. Statistical testing was performed using children with no risk factor as a reference group. Fisher's exact test was used for PICU comparisons, t-test for days of admission. Modified from Hamrin et al. 2020.

	Total N (%)	Age in months Median (IQR)	Days of admission Median (IQR)	PICU N (%)
All included children	2351 (100%)	4.8 (1.7 - 16.4)	3 (2 - 5)	187 (8%)
No risk factor	1718 (73%)	3.1 (1.4 - 9.3)	3 (2 - 5)	108 (6%)
Any risk factor	633 (27%)	16.0 (5.3 - 26.6)	3 (2 - 6)	74 (12%) ***
Recurrent wheezing / Asthma	231 (10%)	19.2 (11.5 - 25.8)	3 (2 - 4) ***	3 (1%) **
Neuromuscular conditions	119 (5%)	25.0 (11.5 - 46.6)	4 (2 - 7)	26 (22%) ***
Preterm infants	134 (6%)	4.1 (1.9 – 8.6)	4 (3 - 6) *	27 (20%) ***
Cardiac conditions	45 (2%)	8.3 (2.3 - 15.1)	3 (2 - 6)	6 (13%)
Oncologic condition / Immunodeficiency	36 (2%)	22.8 (17.6 - 54.6)	5 (2 - 8)	1 (3%)
Chronic lung disease	64 (2.7)	19.5 (5.6 - 36.9)	4 (3 - 6)	11 (17%) ***
Other underlying conditions	10 (0.4)	18.2 (12.2 – 27.5)	4 (3 - 6.5)	
< 6 months of age	1297 (55%)	1.84 (1.1 - 3.3)	4 (2 - 5)	146 (11%) ***

Our results highlight well-known risk factors for severe RSV infection: Prematurity, chronic lung disease and neurologic conditions and young age (<6 months). The first three mentioned risk-groups are already subject to passive immunization with palivizumab and it is a likely explanation for the low representation of children with cardiac malformations [89,262]. One might also consider expanding palivizumab prophylaxis among children with neuromuscular

conditions [85]. Our data suggest that asthma or recurrent wheezing may not be a true risk factor as duration of hospitalization and PICU admission is significantly lower compared to children without risk factors. Even though age < 6 months emerges as a risk factor, the risk of young age in infants may still be underestimated as was also mentioned in results regarding RSV epidemiology. Because the steep drop in risk of requiring hospital admission in the first 3 months of life is 'diluted' by the often-used age span of <6 months. Because incidence rates are typically presented on an annual basis, children born before and after RSV epidemics as well as those without older siblings further 'dilute' the actual risk because they are less exposed to RSV. The heatmap presented in **Figure 29** highlights this issue.

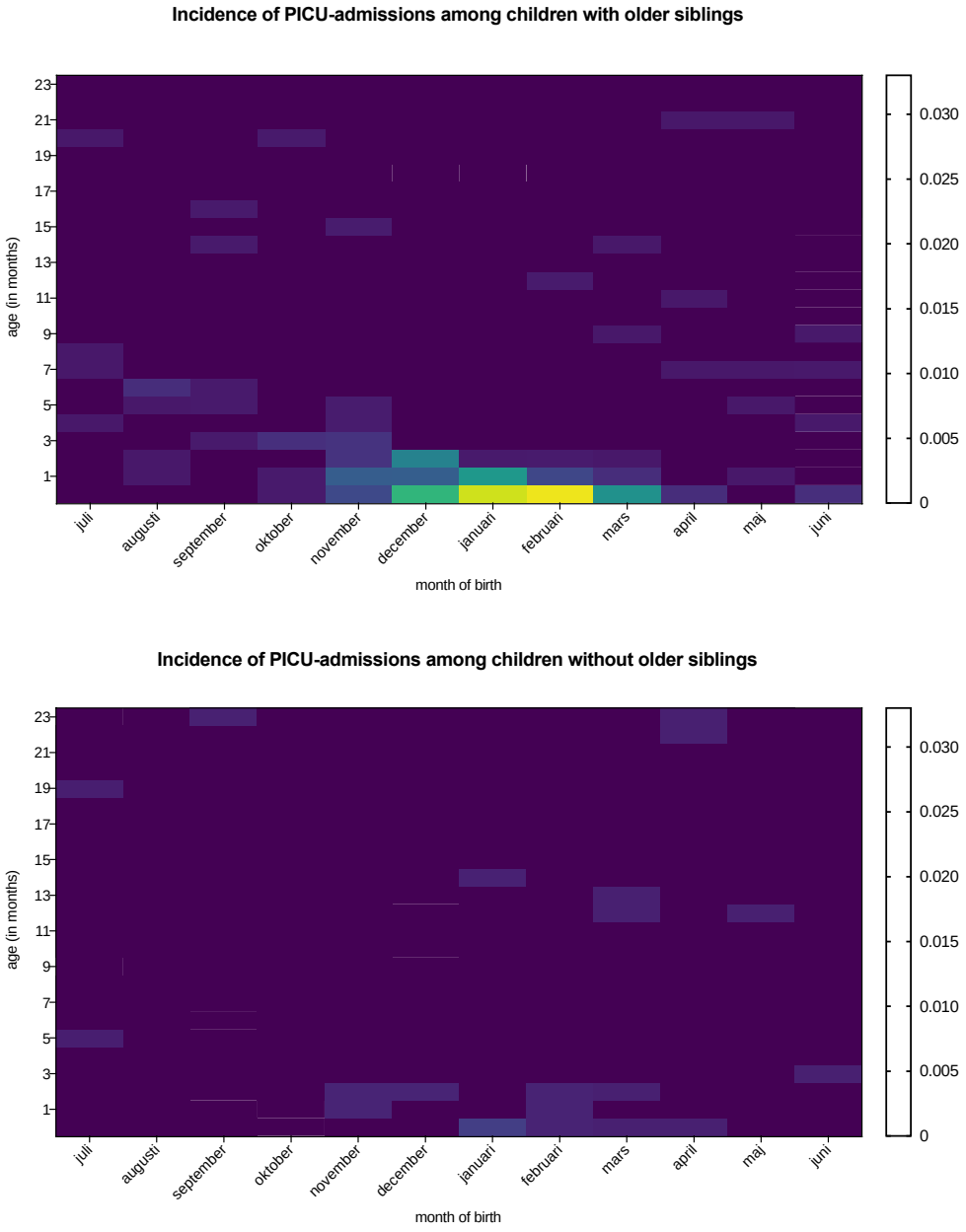

Figure 29: Incidence of PICU admission for children residing in the Northern Stockholm region 2008 – 2016 in relation to age (in months) and month of birth for children with (upper panel) and without (lower panel) older siblings.

The importance of age, month of birth and the presence of siblings is underscored in numbers in **Table 8**. All comparisons are population-based incidence rates. Winter was defined as January – March and summer May – June.

Table 8: Incidence of hospital admission and PICU admission per 100 children. The top row (Infants <3 months of age in winter with siblings) was compared to subsequent rows with Fischer's exact and Odds Ratio (OR) and Confidence Intervals (CI). Data based on hospitalization in Northern Stockholm region 2008 – 2016. Winter defined as January – March, Summer as May – July.

	Hospital admission	OR (CI)	PICU admission	OR (CI)
Infants <3 months of age in winter with siblings	**6.82**		**1.26**	
*Infants <3 months of age **in summer** with siblings*	0.11 ***	55 (24 – 129)	0.06 ***	16 (5 – 48)
*Infants **3-6 months of age** in winter with siblings*	0.22 ***	30 (16 – 57)	0.07 ***	15 (5 – 44)
*Infants <3 months of age in winter **without siblings***	1.63 ***	4.1 (3.1 – 5.6)	0.17 ***	7 (3 – 15)

Our data clearly show the importance of protecting infants <3 months, born in the midst of RSV epidemics from infection. Any measure keeping older siblings home during the first 3 months of infancy has the potential to reduce the risk of infection necessitating hospital admission fourfold. One can appreciate the strain on presumably mothers having a newborn to breastfeed and connect to while keeping a 2-3year old at home. On the other hand, Covid-19 has shown and taught us as a population the importance of social distancing. In this scenario, families would be practicing social isolation to protect their newborn. Furthermore, however short-lived maternal antibodies may be, they would be providing the best protection when it is needed most, i.e. in the first 1-3 months of the newborn. This makes a strong case for maternal RSV vaccination during pregnancy. A single dose of palivizumab may also be considered for infants born in RSV peaks with siblings, but may not be justified from a health economic point of view, given current pricing.

6 CONCLUSIONS

This thesis encompasses several large epidemiological studies on children hospitalized with IFV and RSV infections. These studies address factors determining patterns of epidemics, risk factors for severe disease and complications. It also includes a prospective clinical study investigating a potential marker for severe viral disease.

PGE_2 may be a functional marker for immunopathological processes leading to severe disease in viral RTIs. Our results indicate that the urinary PGE_2 metabolite (u-tPGEM) is elevated in LRTIs in contrast to URTIs. However, further studies are needed to clarify the role of PGE_2 in the immune response and its predictive value in identifying children at risk for severe disease. The value of these efforts should be seen in the light of the potential of other more comprehensive markers of immune responses, such as transcriptome profiling.

Investigating drivers of a specific viral epidemic is challenging because it requires stable conditions with respect to demography, meteorology and climate and circulation of other viruses and time. In a sense, one epidemic season corresponds to one experiment. In the case of RSV with a delayed biennial pattern, one experiment requires observation two epidemic seasons. Changes to any mentioned condition should ideally occur on separate occasions in order to determine their true effect. Our study of the irregular RSV epidemics exemplifies this point as those winters were exceptionally cold, exceptionally many children were born, and all this occurred in the wake of an influenza pandemic. Having said this, the combination of our clinical data underscoring the importance of young age for severe RSV infection and the predictions made by mathematical RSV-models suggest a substantial role for birth rates as a determinant of RSV epidemics.

The ongoing pandemic caused by CoV-SARS-2 has taught us the tremendous impact of viral interference as RSV- and IFV were virtually absent in the winter of 2020-2021[263–265]. In the case of RSV, children's hospitals are now struggling with severe 'recoil' of RSV-infections, most likely due to loss of herd immunity in the previous winter. Advancing our understanding whether viral interference occurred through a viral, immunological or behavioral mechanism would much improve our ability to predict repercussions of future pandemics and to implement effective prevention efforts.

The better we are able to define individuals at risk for severe viral infections, the more we can target both preventions and therapeutic approaches. Examples of this are infants <3 months of age with siblings, born during RSV peaks and children with severe neurological impairments.

Needless to say, there remains much to be learned about pathological processes leading to severe viral LRTIs, drivers of viral epidemics, the viral ecosystem as well as treatment and prevention.

7 POINTS OF PERSPECTIVE

7.1 BIOMARKERS FOR SEVERE INFECTION

Transcriptome analysis is a high-throughput method were several hundreds of microRNAs responsible for gene can be evaluated. The gene regulation pattern reveals a which mechanisms, cells and pathways are involved the immunological responses to infections or other inflammatory responses. In the case of RSV, this method has been able to differentiate the immune response patterns of infants with severe disease from mild disease [266]. An important subsequent step would be to follow infants with consecutive sampling during the course of a disease to further the understanding of pathogenesis and to identify potential targets for treatment.

7.2 MODELING AND PREDICTING VIRAL EPIDEMICS

Mathematical models based on the previously described SIRS have already been established for RSV as previously mentioned. They typically depend on birth rates as a single variable, with other parameters fixed, such as seasonal forcing by seasonal meteorologic conditions. In addition, several models contain assumptions perhaps less relevant to viral infections in childhood. Mathematicians Virginia Pitzer at Yale University and Alexandra Hogan at Imperial College have generously shared their SIRS models for RSV epidemics. We are currently seeking the help of mathematicians at Stockholm University to assist us in adapting these programmed models to fit our circumstances. For instance, we would like to replace a fixed parameter for seasonal forcing with measured temperatures and introduce a variable to simulate viral interference. This may allow us to test changes in one epidemic driver at once on a theoretical level. Hopefully this could contribute to better predictions of viral epidemics which could improve planning of health care resources.

7.3 UNDERSTANDING THE ECOLOGY OF RESPIRATORY VIRUSES

Very much related to the previous point, the quality of prediction models is highly dependent on continued surveillance of hospital cases with consistent sampling. Ideally, concomitant sampling should be conducted among relevant cohorts in healthy controls in order to assess the true burden and kinetics of viral epidemics. Furthermore, viral testing should capture a broad spectrum of respiratory viruses to further the understanding of the respiratory virus ecosystem.

7.4 DISEASE PREVENTION

Our data identify a defined risk-group where preventative measures in a limited time could potentially reduce the number of infants succumbing to severe RSV disease substantially. Indirectly, this would also benefit children with other diseases as more health resources will be available to attend to them. One possibility would be an intervention study informing and recommending parents expecting their second or subsequent child during a time when RSV epidemics to keep siblings attending daycare at home for the first three months. The information should be given reasonably early during pregnancy visits to provide the parents with the opportunity to plan their parental leaves accordingly. Needless to say, keeping a toddler at home with a newborn will not be a reasonable option for all families. The effect could be measured in total incidence rates and/or incidence rates for a targeted intervention group compared to historical and perhaps geographic controls. Of course, the current perturbation of RSV epidemics complicates the notion of historical controls and would require the study the potential effect over several seasons to avoid confounding by the biennial pattern.

8 ACKNOWLEDGEMENTS

To my wife Thirza. When I registered as a PhD student, we had no children and now our sons are 9,7 and 5 years old. My love, behind every sentence written in this book, there is some kind of sacrifice or extra effort you have made to keep the household running, all the boys' activities going and compensating for mental absence when I was too focused on research. I am eternally grateful.

To my boys Harry, Hilmer and Casper for love, humor and constantly proving that the quest for knowledge is in our nature.

To Anna Nilsson, my main supervisor, who is a true role model both in research and as a pediatric oncologist. You made it your task to get me through my PhD studies with tremendous amount of patience, encouragement and by simply believing in me.

To Margareta Eriksson, for generously involving me in her research from the start. You are the motor behind all the epidemiological studies. You inspire me with your insatiable curiosity and relentless quest to improve our care in pediatric infectious diseases.

To Anna-Lena Spetz, for giving me the opportunity to test my wings in your laboratory with your talented and pleasant team. While struggling with my experiments, I was learning how to be a researcher and those lessons have stretched well into my recent epidemiological studies.

To Eric Herlenius, the catalyst of my PhD studies. I am proud of accomplishing the PGE_2-study together with you.

To Lars Björk, for teaching me many 'nuts and bolts' in clinical research and sharing your great knowledge of immunology with me.

To Rutger Bennet, for being my secret supervisor as you have been involved in all my epidemiological studies. You have generously shared your knowledge of data analysis, pediatric diseases and your great sense of humor.

To all parents who have consented to sampling of their child, even as they were in the stressful situation of having to stay at the hospital with their sick child.

To all the former staff at Q80, for helping me recruit patients and collect samples in the midst of an RSV epidemic. You always made me feel at home at your ward and also allowed me to grow as a clinician.

To Rickard Markgren, then a medical student, for getting involved in recruiting patients and collecting urinary samples while providing great company.

To Peter Järver and Aleksandra Dondalska, my room mates at the Spetz lab, for teaching me proper laboratory work and making every day enjoyable with great humor, music and companionship.

To Ida Hed Myrberg, for digging into mathematical modeling with me and trying to make sense of incomprehensible computer code.

To Joachim Luthander, my clinical supervisor in pediatric training, for all your encouragement in the clinic, in research and for sharing your insights about work-life balance with me.

To Petter Svenberg, for molding me into a pediatric oncologist through teaching and setting an inspiring example. That, and your renowned sense of humor makes it a joy to work with you.

To Niklas Pal, for generously mentoring me as I am entering the field of solid tumors, for sharing your wisdom, experience and providing me with great tips for vegan restaurants.

To Pernilla Grillner and Susanna Ranta for giving me the time to finish my thesis.

To all my incredibly knowledgeable, dedicated friends/colleagues at the pediatric oncology department. And yes, that includes our friends at hematology.

To all the nurses at the pediatric oncology department for the pleasure of sharing the commitment to helping severely ill children.

To my mother, for all the love and raising me with a conviction that this is a world where kindness and dedication prevails. For being the glue of the family.

To my father, for an adventurous childhood and for challenging me to find myself.

To my siblings Maria, Lotta, Lina and Stefan, for love, friendship and always having my back. All the effort we have put into teasing each other has eventually created a soothing revelation that we don’t have to take ourselves too seriously.

To all my dear friends from high school, my medical studies and spread out in different countries: for fully appreciating the accomplishment of finalizing this thesis by a procrastinating, disorganized mind that is mostly in a state somewhere in between daydreaming, sleepy or asleep. Thank you for providing a safe haven for all my shortcomings and quirks.

To Kalle Grinnemo, Oscar Simonsson, Matthias Cobascio and Thomas Fux: Thank you for bringing truth to light.

This research has been very generously sponsored by Region Stockholm by entering me into the research residency program (*Forskar-ST*). I am also grateful for the funding I received from *Sällskapet Barnavård*.

9 REFERENCES

1. Collaborators, G. 2016 L. R. I. *et al.* Estimates of the global, regional, and national morbidity, mortality, and aetiologies of lower respiratory infections in 195 countries, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Infect Dis* **18**, 1191–1210 (2018).

2. Shi, T. *et al.* Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in young children in 2015: a systematic review and modelling study. *Lancet* **390**, 946–958 (2017).

3. Nair, H. *et al.* Global burden of respiratory infections due to seasonal influenza in young children: a systematic review and meta-analysis. *Lancet* **378**, 1917–1930 (2011).

4. 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. *Journal of global health* **5**, 010408 (2015).

5. Rhedin, S. *et al.* Respiratory viruses associated with community-acquired pneumonia in children: matched case-control study. *Thorax* **70**, 847–853 (2015).

6. Cui, X. *et al.* A systematic review and meta-analysis of children with coronavirus disease 2019 (COVID-19). *J Med Virol* **93**, 1057–1069 (2021).

7. Diorio, C. *et al.* Multisystem inflammatory syndrome in children and COVID-19 are distinct presentations of SARS-CoV-2. *J Clin Invest* **130**, 5967–5975 (2020).

8. Eriksson, M., Bennet, R., Rotzen-Ostlund, M., Sydow, M. von & Wirgart, B. Population-based rates of severe respiratory syncytial virus infection in children with and without risk factors, and outcome in a tertiary care setting. *Acta Paediatr* **91**, 593–598 (2002).

9. Storch, G. A. *Chapter 17: Diagnostic Virology, Fields Virology, 5th Edition*. (Lipincott Williams & Wilkins, 2007).

10. Villenave, R. *et al.* In vitro modeling of respiratory syncytial virus infection of pediatric bronchial epithelium, the primary target of infection in vivo. *Proceedings of the National Academy of Sciences* **109**, 5040–5045 (2012).

11. Lemon, K. *et al.* Recombinant Subgroup B Human Respiratory Syncytial Virus Expressing Enhanced Green Fluorescent Protein Efficiently Replicates in Primary Human Cells and Is Virulent in Cotton Rats. *J Virol* **89**, 2849–2856 (2015).

12. Östlund, M. R. Respiratory virus infections in Stockholm during seven seasons: a retrospective study of laboratory diagnosis. *Scandinavian Journal of Infectious Diseases* (2004) doi:10.1080/00365540410015295.

13. Tiveljung-Lindell, A. *et al.* Development and implementation of a molecular diagnostic platform for daily rapid detection of 15 respiratory viruses. *Journal of Medical Virology* **81**, 167–175 (2009).

14. Liu, C. Rapid Diagnosis of Human Influenza Infection from Nasal Smears by Means of Fluorescein-Labeled Antibody. *Proceedings of the Society for Experimental Biology and Medicine* **92**, 883–887 (1956).

15. Weinberg, A. & Walker, M. L. Evaluation of Three Immunoassay Kits for Rapid Detection of Influenza Virus A and B. *Clin Diagn Lab Immun* **12**, 367–370 (2005).

16. Ohm-Smith, M. J., Nassos, P. S. & Haller, B. L. Evaluation of the Binax NOW, BD Directigen, and BD Directigen EZ Assays for Detection of Respiratory Syncytial Virus. *Journal of Clinical Microbiology* **42**, 2996–2999 (2004).

17. Chen, G.-W. & Shih, S.-R. Genomic Signatures of Influenza A Pandemic (H1N1) 2009 Virus. *Emerg Infect Dis* **15**, 1897–1903 (2009).

18. Arias, C. F. *et al.* Molecular anatomy of 2009 influenza virus A (H1N1). *Archives of medical research* **40**, 643–654 (2009).

19. Ripa, T. & Nilsson, P. A. A Chlamydia trachomatis Strain With a 377-bp Deletion in the Cryptic Plasmid Causing False-Negative Nucleic Acid Amplification Tests. *Sex Transm Dis* **34**, 255–256 (2007).

20. Mukhopadhyay, R. DNA sequencers: the next generation. *Anal Chem* **81**, 1736–1740 (2009).

21. Vemula, S. V. *et al.* Current Approaches for Diagnosis of Influenza Virus Infections in Humans. *Viruses* **8**, 96 (2016).

22. Pangesti, K. N. A., Ghany, M. A. E., Walsh, M. G., Kesson, A. M. & Hill-Cawthorne, G. A. Molecular epidemiology of respiratory syncytial virus. *Rev Med Virol* **28**, e1968 (2018).

23. Wang, H. *et al.* The genetic sequence, origin, and diagnosis of SARS-CoV-2. *Eur J Clin Microbiol* **39**, 1629–1635 (2020).

24. Abbas, A. K., Lichtman, A. H. & Pillai, S. *Basic Immunology: Chapter 2 - Innate Immunity, The Early Defense Against Infections*. (Elsevier Saunders, 2012).

25. Krammer, F. The human antibody response to influenza A virus infection and vaccination. *Nat Rev Immunol* **19**, 383–397 (2019).

26. Dowell, S. F. *et al.* Respiratory Syncytial Virus Is an Important Cause of Community-Acquired Lower Respiratory Infection among Hospitalized Adults. *J Infect Dis* **174**, 456–462 (1996).

27. Storch, G. A. Chapter 17: Diagnostic Virology, Fields Virology 5th Edition. in (2007).

28. Deeks, J. J. *et al.* Antibody tests for identification of current and past infection with SARS-CoV-2. *Cochrane Db Syst Rev* **2020**, CD013652 (2020).

29. Wallace, L. A. *et al.* Virological surveillance of influenza-like illness in the community using PCR and serology. *J Clin Virol* **31**, 40–45 (2004).

30. 2, A. F. Respiratory Syncytial Virus Infection in Adults. *Semin Respir Crit Care Med* **28**, 171–181 (2007).

31. Perez, L. Acute phase protein response to viral infection and vaccination. *Arch Biochem Biophys* **671**, 196–202 (2019).

32. Covington, E. W., Roberts, M. Z. & Dong, J. Procalcitonin Monitoring as a Guide for Antimicrobial Therapy: A Review of Current Literature. *Pharmacother J Hum Pharmacol Drug Ther* **38**, 569–581 (2018).

33. Simon, L., Gauvin, F., Amre, D. K., Saint-Louis, P. & Lacroix, J. Serum Procalcitonin and C-Reactive Protein Levels as Markers of Bacterial Infection: A Systematic Review and Meta-analysis. *CID* 206–217 (2004) doi:10.1086/421997.

34. Ahout, I. M. L. *et al.* Prospective observational study in two Dutch hospitals to assess the performance of inflammatory plasma markers to determine disease severity of viral respiratory tract infections in children. *Bmj Open* **7**, e014596 (2017).

35. Engelmann, I. *et al.* Diagnosis of Viral Infections Using Myxovirus Resistance Protein A (MxA). *Pediatrics* **135**, e985–e993 (2015).

36. Ma, X. *et al.* Can we distinguish respiratory viral infections based on clinical features? A prospective pediatric cohort compared to systematic literature review. *Rev Med Virol* **28**, e1997 (2018).

37. *Nelson textbook of pediatrics, 18th edition*. (Saunders Elsevier, 2007).

38. *Principles and Practice of Infectious Diseases, 8th Edition*. (Saunders, Elsevier, 2015). doi:https://doi.org/10.1016/C2012-1-00075-6.

39. Fernandes, R. M. *et al.* Glucocorticoids for acute viral bronchiolitis in infants and young children. *Cochrane Db Syst Rev* **6**, CD004878 (2013).

40. Karampatsas, K., Kong, J. & Cohen, J. Bronchiolitis: an update on management and prophylaxis. *Brit J Hosp Med* **80**, 278–284 (2019).

41. Tregoning, J. S. & Schwarze, J. Respiratory viral infections in infants: causes, clinical symptoms, virology, and immunology. *Clinical Microbiology Reviews* **23**, 74–98 (2010).

42. Roux, D. M. le & Zar, H. J. Community-acquired pneumonia in children — a changing spectrum of disease. *Pediatr Radiol* **47**, 1392–1398 (2017).

43. Sweden, P. H. A. of. Pediatric vaccinations. https://www.folkhalsomyndigheten.se/smittskydd-beredskap/vaccinationer/vacciner-a-o (2020).

44. Zar, H. J. *et al.* Aetiology of childhood pneumonia in a well vaccinated South African birth cohort: a nested case-control study of the Drakenstein Child Health Study. *Lancet Respir Medicine* **4**, 463–472 (2016).

45. Jain, S. *et al.* Community-Acquired Pneumonia Requiring Hospitalization among U.S. Children. *New Engl J Medicine* **372**, 835–845 (2015).

46. Eriksson, M., Nilsson, A. & Bennet, R. Changing diagnosis coding routines may confound the results of longitudinal childhood pneumonia studies. *Acta Paediatr* **106**, 1825–1829 (2017).

47. Chung, B. & Wong, V. Relationship between five common viruses and febrile seizure in children. *Arch Dis Child* **92**, 589 (2007).

48. Wickström, R., Fowler, Å., Bogdanovic, G., Bennet, R. & Eriksson, M. Review of the aetiology, diagnostics and outcomes of childhood encephalitis from 1970 to 2009. *Acta Paediatr* **106**, 463–469 (2017).

49. GRANEROD, J. *et al.* Causality in acute encephalitis: defining aetiologies. *Epidemiol Infect* **138**, 783–800 (2010).

50. Britton, P. N. *et al.* Causes and Clinical Features of Childhood Encephalitis: A Multicenter, Prospective Cohort Study. *Clin Infect Dis* **70**, 2517–2526 (2019).

51. Wolf, D. G. *et al.* Comparison of Human Metapneumovirus, Respiratory Syncytial Virus and Influenza A Virus Lower Respiratory Tract Infections in Hospitalized Young Children. *Pediatric Infect Dis J* **25**, 320–324 (2006).

52. Minodier, L. *et al.* Prevalence of gastrointestinal symptoms in patients with influenza, clinical significance, and pathophysiology of human influenza viruses in faecal samples: what do we know? *Virol J* **12**, 215 (2015).

53. Agyeman, P., Duppenthaler, A., Heininger, U. & Aebi, C. Influenza-associated myositis in children. *Infection* **32**, 199–203 (2004).

54. Freund, M. W., Kleinveld, G., Krediet, T. G., Loon, A. M. van & Verboon-Maciolek, M. A. Prognosis for neonates with enterovirus myocarditis. *Archives Dis Child - Fetal Neonatal Ed* **95**, F206 (2010).

55. Madjid, M., Safavi-Naeini, P., Solomon, S. D. & Vardeny, O. Potential Effects of Coronaviruses on the Cardiovascular System. *JAMA Cardiology* 1–10 (2020) doi:10.1001/jamacardio.2020.1286.

56. Rothberg, M. B., Haessler, S. D. & Brown, R. B. Complications of Viral Influenza. *The American Journal of Medicine* **121**, 258–264 (2008).

57. Brodin, P. & Davis, M. M. Human immune system variation. *Nat Rev Immunol* **17**, 21–29 (2017).

58. Ygberg, S. & Nilsson, A. The developing immune system - from foetus to toddler. *Acta Paediatrica* **101**, 120–127 (2012).

59. Shi, T. *et al.* Risk factors for respiratory syncytial virus associated with acute lower respiratory infection in children under five years: Systematic review and meta–analysis. *J Glob Health* **5**, 020416 (2015).

60. Ma, H.-Y. *et al.* Risk factors associated with severe influenza virus infections in hospitalized children during the 2013 to 2014 season. *Journal of Microbiology, Immunology and Infection* 1–7 (2015) doi:10.1016/j.jmii.2015.05.015.

61. Dawood, F. S. *et al.* Burden of seasonal influenza hospitalization in children, United States, 2003 to 2008. *The Journal of Pediatrics* **157**, 808–814 (2010).

62. Gill, P. J. *et al.* Identification of children at risk of influenza-related complications in primary and ambulatory care: a systematic review and meta-analysis. *Lancet Respir Medicine* **3**, 139–149 (2015).

63. Shane, A. L., Sánchez, P. J. & Stoll, B. J. Neonatal sepsis. *Lancet* **390**, 1770–1780 (2017).

64. Bender, J. M. *et al.* Influenza Virus Infection in Infants Less Than Three Months of Age. *Pediatric Infect Dis J* **29**, 6–9 (2010).

65. Stocker, M. *et al.* Procalcitonin-guided decision making for duration of antibiotic therapy in neonates with suspected early-onset sepsis: a multicentre, randomised controlled trial (NeoPIns). *Lancet* **390**, 871–881 (2017).

66. Larsen, J. W. W. *et al.* Hospitalizations for infections by age and sex: register-based study of Danish children 1977–2014. *Infect Dis-nor* **52**, 1–10 (2019).

67. Yang, T. O., Huang, W.-T., Chen, M.-H., Huang, K.-Y. A. & Chen, P.-C. Sex differences in common childhood infections in Taiwan. *Int J Infect Dis* **75**, 115–117 (2018).

68. Orimadegun, A. E., Adepoju, A. A. & Myer, L. A Systematic Review and Meta-analysis of Sex Differences in Morbidity and Mortality of Acute Lower Respiratory Tract Infections Among African Children. *J Pediatrics Rev* **8**, 65–78 (2020).

69. Fritz, C. Q. *et al.* Prevalence, Risk Factors, and Outcomes of Bacteremic Pneumonia in Children. *Pediatrics* **144**, e20183090 (2019).

70. Shi, T. *et al.* Mortality risk factors in children with severe influenza virus infection admitted to the pediatric intensive care unit. *Medicine* **98**, e16861 (2019).

71. Muenchhoff, M. & Goulder, P. J. R. Sex Differences in Pediatric Infectious Diseases. *Journal of Infectious Diseases* **209**, S120–S126 (2014).

72. Nicolai, A. *et al.* Risk Factors for Virus-induced Acute Respiratory Tract Infections in Children Younger Than 3 Years and Recurrent Wheezing at 36 Months Follow-Up After Discharge. *Pediatric Infect Dis J* **36**, 179–183 (2017).

73. Moe, N. *et al.* Comparing Human Metapneumovirus and Respiratory Syncytial Virus: Viral Co-Detections, Genotypes and Risk Factors for Severe Disease. *PloS one* **12**, e0170200 (2017).

74. Hardelid, P., Verfuerden, M., McMenamin, J. & Gilbert, R. Risk factors for admission to hospital with laboratory-confirmed influenza in young children: birth cohort study. *Eur Respir J* **50**, 1700489 (2017).

75. Haerskjold, A. *et al.* Risk Factors for Hospitalization for Respiratory Syncytial Virus Infection. *Pediatric Infect Dis J* **35**, 61–65 (2016).

76. Simoes, E. A. F. Environmental and demographic risk factors for respiratory syncytial virus lower respiratory tract disease. *J Pediatrics* **143**, 118–126 (2003).

77. Young, M. & Smitherman, L. Socioeconomic Impact of RSV Hospitalization. *Infect Dis Ther* **10**, 35–45 (2021).

78. Andreas, N. J., Kampmann, B. & Le-Doare, K. M. Human breast milk: A review on its composition and bioactivity. *Early Hum Dev* **91**, 629–635 (2015).

79. Tarrant, M., Kwok, M.-K., Lam, T.-H., Leung, G. M. & Schooling, C. M. Breast-feeding and Childhood Hospitalizations for Infections. *Epidemiology* **21**, 847–854 (2010).

80. McEvoy, C. T. & Spindel, E. R. Pulmonary Effects of Maternal Smoking on the Fetus and Child: Effects on Lung Development, Respiratory Morbidities, and Life Long Lung Health. *Paediatr Respir Rev* **21**, 27–33 (2017).

81. PEAT, J. K., Keena, V., Harakeh, Z. & Marks, G. Parental smoking and respiratory tract infections in children. *Paediatr Respir Rev* **2**, 207–213 (2001).

82. Nhung, N. T. T. *et al.* Short-term association between ambient air pollution and pneumonia in children: A systematic review and meta-analysis of time-series and case-crossover studies. *Environ Pollut* **230**, 1000–1008 (2017).

83. Romieu, I., Samet, J. M., Smith, K. R. & Bruce, N. Outdoor Air Pollution and Acute Respiratory Infections Among Children in Developing Countries. *J Occup Environ Med* **44**, 640–649 (2002).

84. Panitch, H. B. Respiratory Implications of Pediatric Neuromuscular Disease. *Respir Care* **62**, 826–848 (2017).

85. Simon, A. A., Prusseit, J. J. & Müller, A. A. Respiratory syncytial virus infection in children with neuromuscular impairment. *Open Microbiology Journal* **5**, 155–158 (2010).

86. Keren, R. *et al.* Neurological and Neuromuscular Disease as a Risk Factor for Respiratory Failure in Children Hospitalized With Influenza Infection. *Jama* **294**, 2188–2194 (2005).

87. Solís-García, G. *et al.* Neurologic Complications in Children Hospitalized With Influenza Infections: Prevalence, Risk Factors and Impact on Disease Severity. *Pediatr Infect Dis J* **39**, 789–793 (2020).

88. Carman, K. B. *et al.* Viral etiological causes of febrile seizures for respiratory pathogens (EFES Study). *Hum Vacc Immunother* **15**, 496–502 (2018).

89. Groothuis, J. R., Fryzek, J. P., Makari, D., Steffey, D. & Martone, W. J. Respiratory syncytial virus hospitalization trends in infants with chronic lung disease of infancy, 1998-2008. *Clinical epidemiology* **3**, 245–250 (2011).

90. McLean, H. Q. *et al.* Serious outcomes of medically attended, laboratory-confirmed influenza illness among school-aged children with and without asthma, 2007-2018. *Influenza Other Resp* **14**, 173–181 (2020).

91. Lo, M. S. *et al.* The impact of RSV, adenovirus, influenza, and parainfluenza infection in pediatric patients receiving stem cell transplant, solid organ transplant, or cancer chemotherapy. *Pediatr Transplant* **17**, 133–143 (2013).

92. Chemaly, R. F. *et al.* Respiratory Syncytial Virus Infections in Children With Cancer. *J Pediatric Hematology Sol Oncol* **36**, e376–e381 (2014).

93. Carr, S. B. *et al.* Clinical and Demographic Characteristics of Seasonal Influenza in Pediatric Patients With Cancer. *Pediatric Infect Dis J* **31**, e202–e207 (2012).

94. Köker, S. A. *et al.* A 3-Year Retrospective Study of the Epidemiology of Acute Respiratory Viral Infections in Pediatric Patients With Cancer Undergoing Chemotherapy. *J Pediatric Hematology Oncol* **41**, e242–e246 (2018).

95. Söderman, M. *et al.* Frequent Respiratory Viral Infections in Children with Febrile Neutropenia - A Prospective Follow-Up Study. *PloS one* **11**, e0157398 (2016).

96. Dawood, F. S. *et al.* Complications and associated bacterial coinfections among children hospitalized with seasonal or pandemic influenza, United States, 2003-2010. *The Journal of infectious diseases* **209**, 686–694 (2014).

97. Kutter, J. S., Spronken, M. I., Fraaij, P. L., Fouchier, R. A. & Herfst, S. Transmission routes of respiratory viruses among humans. *Curr Opin Virol* **28**, 142–151 (2018).

98. Treanor, J. J. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. 2000-2024.e6 (2015) doi:10.1016/b978-1-4557-4801-3.00167-3.

99. Wright, P. F., Neumann, G. & Kawaoka, Y. Chapter 48: Orthomyxoviruses, Fields Virology 5th Edition. in vol. II 1693–1740 (2007).

100. Wilking, A. N., Elliott, E., Garcia, M. N., Murray, K. O. & Munoz, F. M. Central nervous system manifestations in pediatric patients with influenza A H1N1 infection during the 2009 pandemic. *Pediatric neurology* **51**, 370–376 (2014).

101. Coffin, S. E. *et al.* Incidence, Complications, and Risk Factors for Prolonged Stay in Children Hospitalized With Community-Acquired Influenza. *Pediatrics* **119**, 740–748 (2007).

102. CDC: How Influenza (Flu) Vaccines Are Made. https://www.cdc.gov/flu/prevent/how-fluvaccine-made.htm (2021).

103. Grohskopf, L. A. *et al.* Prevention and Control of Seasonal Influenza with Vaccines: Recommendations of the Advisory Committee on Immunization Practices — United States, 2019–20 Influenza Season. *MMWR Recomm Rep.* (2019) doi:10.15585/mmwr.rr6803a1.

104. Trombetta, C. M., Gianchecci, E. & Montomoli, E. Influenza vaccines: Evaluation of the safety profile. *HUMAN VACCINES & IMMUNOTHERAPEUTICS* **14**, 657–670 (2017).

105. Ortqvist, A. *et al.* Long term effectiveness of adjuvanted influenza A(H1N1)pdm09 vaccine in children. *Vaccine* **33**, 2558–2561 (2015).

106. Sarkanen, T., Alakuijala, A., Julkunen, I. & Partinen, M. Narcolepsy Associated with Pandemrix Vaccine. *Curr Neurol Neurosci* **18**, 43 (2018).

107. Sweden, P. H. A. of. Recommendations for pediatric influenza vaccinations in Sweden. https://www.folkhalsomyndigheten.se/smittskydd-beredskap/vaccinationer/fragor-och-svar/barn-och-sasongsinfluensavaccin/ (2019).

108. Hayden, F. G. *et al.* Baloxavir Marboxil for Uncomplicated Influenza in Adults and Adolescents. *New Engl J Med* **379**, 913–923 (2018).

109. Noshi, T. *et al.* In vitro characterization of baloxavir acid, a first-in-class cap-dependent endonuclease inhibitor of the influenza virus polymerase PA subunit. *Antivir Res* **160**, 109–117 (2018).

110. Esposito, S. & Principi, N. Oseltamivir for influenza infection in children: risks and benefits. *Expert Rev Resp Med* **10**, 79–87 (2015).

111. LI-KIM-MOY, J. *et al.* Influenza hospitalizations in Australian children. *Epidemiol Infect* **145**, 1451–1460 (2017).

112. Yu, J. *et al.* Respiratory Syncytial Virus Seasonality, Beijing, China, 2007–2015 - Volume 25, Number 6—June 2019 - Emerging Infectious Diseases journal - CDC. *Emerg Infect Dis* **25**, 1127–1135 (2019).

113. White, L. J., Waris, M., Cane, P. A., Nokes, D. J. & Medley, G. F. 15816153.pdf. *Epidemiol. Infect.* 279–289 (2004) doi:10.1017/s095026880400345.

114. Obando-Pacheco, P. *et al.* Respiratory Syncytial Virus Seasonality: A Global Overview. *The Journal of infectious diseases* **217**, 1356–1364 (2018).

115. Baker, R. E. *et al.* Epidemic dynamics of respiratory syncytial virus in current and future climates. *Nature communications* **10**, 5512 (2019).

116. Yusuf, S. *et al.* The relationship of meteorological conditions to the epidemic activity of respiratory syncytial virus. *Epidemiology and infection* **135**, 1077–1090 (2007).

117. Sloan, C. *et al.* The impact of temperature and relative humidity on spatiotemporal patterns of infant bronchiolitis epidemics in the contiguous United States. *Health & place* **45**, 46–54 (2017).

118. Spurling, G. K., Doust, J., Mar, C. B. D. & Eriksson, L. Antibiotics for bronchiolitis in children. *Cochrane Db Syst Rev* CD005189 (2011) doi:10.1002/14651858.cd005189.pub3.

119. Stein, R. *et al.* Respiratory syncytial virus in early life and risk of wheeze and allergy by age 13 years. *Lancet* **354**, 541–545 (1999).

120. Simões, E. A. F. *et al.* Past, Present and Future Approaches to the Prevention and Treatment of Respiratory Syncytial Virus Infection in Children. *Infectious Diseases and Therapy* **7**, 87–120 (2018).

121. Piedimonte, G. RSV infections: State of the art. *Cleveland Clinic journal of medicine* **82**, S13-8 (2015).

122. KIM, H. W. *et al.* Respiratory Syncytial Virus Disease in Infants Despite Prior Administration of Antigenic Inactivated Vaccine. *American Journal of Epidemiology* **89**, 422- (1969).

123. Boyoglu-Barnum, S., Chirkova, T. & Anderson, L. J. Biology of Infection and Disease Pathogenesis to Guide RSV Vaccine Development. *Front Immunol* **10**, 1675 (2019).

124. Mazur, N. I. *et al.* The respiratory syncytial virus vaccine landscape: lessons from the graveyard and promising candidates. *Lancet Infect Dis* **18**, e295–e311 (2018).

125. Mejias, A., Rodríguez-Fernández, R., Oliva, S., Peeples, Mark. E. & Ramilo, O. The Journey to an RSV Vaccine. *Ann Allergy Asthma Immunol* **125**, 36–46 (2020).

126. Group, T. Im.-R. S. Palivizumab, a humanized respiratory syncytial virus monoclonal antibody, reduces hospitalization from respiratory syncytial virus infection in high-risk infants. The IMpact-RSV Study Group. *PEDIATRICS* **102**, 531–537 (1998).

127. Hoogen, B. G. van den *et al.* A newly discovered human pneumovirus isolated from young children with respiratory tract disease. *Nature medicine* **7**, 719–724 (2001).

128. Graaf, M. de, Osterhaus, A. D. M. E., Fouchier, R. A. M. & Holmes, E. C. Evolutionary dynamics of human and avian metapneumoviruses. *J Gen Virol* **89**, 2933–2942 (2008).

129. Gröndahl, B. *et al.* The 2009 pandemic influenza A(H1N1) coincides with changes in the epidemiology of other viral pathogens causing acute respiratory tract infections in children. *Infection* **42**, 303–308 (2014).

130. Rafiefard, F., Yun, Z. & Örvell, C. Epidemiologic characteristics and seasonal distribution of human metapneumovirus infections in five epidemic seasons in Stockholm, Sweden, 2002–2006. *J Med Virol* **80**, 1631–1638 (2008).

131. Falsey, A. R. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. 1961-1966.e2 (2015) doi:10.1016/b978-1-4557-4801-3.00161-2.

132. Branche, A. & Falsey, A. Parainfluenza Virus Infection. *Semin Resp Crit Care* **37**, 538–554 (2016).

133. Ison, M. G. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. 1937-1941.e2 (2015) doi:10.1016/b978-1-4557-4801-3.00158-2.

134. Benten, I. V. *et al.* Predominance of rhinovirus in the nose of symptomatic and asymptomatic infants. *Pediatr Allergy Immu* **14**, 363–370 (2003).

135. Jacobs, S. E., Lamson, D. M., George, K. S. & Walsh, T. J. Human rhinoviruses. *Clinical Microbiology Reviews* **26**, 135–162 (2013).

136. Turner, R. B. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. 748-752.e2 (2015) doi:10.1016/b978-1-4557-4801-3.00058-8.

137. Turner, R. B. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. 2113-2121.e2 (2015) doi:10.1016/b978-1-4557-4801-3.00177-6.

138. Witsø, E. *et al.* High Prevalence of Human Enterovirus A Infections in Natural Circulation of Human Enteroviruses ▿ †. *J Clin Microbiol* **44**, 4095–4100 (2006).

139. Lee, B. E. *et al.* Paediatric Investigators Collaborative Network on Infections in Canada (PICNIC) study of aseptic meningitis. *Bmc Infect Dis* **6**, 68–68 (2006).

140. Crom, S. C. M. de, Rossen, J. W. A., Furth, A. M. van & Obihara, C. C. Enterovirus and parechovirus infection in children: a brief overview. *Eur J Pediatr* **175**, 1023–1029 (2016).

141. Romero, J. R. & Modlin, J. F. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. 2066-2072.e2 (2015) doi:10.1016/b978-1-4557-4801-3.00172-7.

142. Romero, J. R. & Modlin, J. F. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. 2080-2090.e4 (2015) doi:10.1016/b978-1-4557-4801-3.00174-0.

143. Mao, Q., Wang, Y., Bian, L., Xu, M. & Liang, Z. EV-A71 vaccine licensure: a first step for multivalent enterovirus vaccine to control HFMD and other severe diseases. *Emerg Microbes Infec* **5**, e75 (2016).

144. McIntosh, K. & Perlman, S. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. 1928-1936.e2 (2015) doi:10.1016/b978-1-4557-4801-3.00157-0.

145. Fung, T. S. & Liu, D. X. Human Coronavirus: Host-Pathogen Interaction. *Annu Rev Microbiol* **73**, 1–29 (2019).

146. Rajapakse, N. & Dixit, D. Human and novel coronavirus infections in children: a review. *Paediatr Int Child H* **41**, 36–55 (2020).

147. Radia, T. *et al.* Multi-system Inflammatory Syndrome in Children & Adolescents (MIS-C): A Systematic Review of Clinical features and presentation. *Paediatr Respir Rev* **38**, 51–57 (2020).

148. Alsohime, F., Temsah, M.-H., Al-Nemri, A. M., Somily, A. M. & Al-Subaie, S. COVID-19 infection prevalence in pediatric population: Etiology, clinical presentation, and outcome. *J Infect Public Heal* **13**, 1791–1796 (2020).

149. Eberhardt, C. S. & Siegrist, C. Is there a role for childhood vaccination against COVID-19? *Pediatr Allergy Immu* **32**, 9–16 (2021).

150. Lynch, J. & Kajon, A. Adenovirus: Epidemiology, Global Spread of Novel Serotypes, and Advances in Treatment and Prevention. *Semin Resp Crit Care* **37**, 586–602 (2016).

151. Calvo, C. *et al.* Eight Year Prospective Study of Adenoviruses Infections in Hospitalized Children. Comparison with Other Respiratory Viruses. *PloS one* **10**, e0132162 (2015).

152. Muller, W. J. *et al.* Clinical and In Vitro Evaluation of Cidofovir for Treatment of Adenovirus Infection in Pediatric Hematopoietic Stem Cell Transplant Recipients. *Clin Infect Dis* **41**, 1812–1816 (2005).

153. Rhee, E. G. & Barouch, D. H. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. 1787-1793.e2 (2015) doi:10.1016/b978-1-4557-4801-3.00145-4.

154. Allander, T. *et al.* Cloning of a human parvovirus by molecular screening of respiratory tract samples. *P Natl Acad Sci Usa* **102**, 12891–12896 (2005).

155. Schildgen, O. *et al.* Human bocavirus: passenger or pathogen in acute respiratory tract infections? *Clinical Microbiology Reviews* **21**, 291-contents (2008).

156. Torow, N., Marsland, B. J., Hornef, M. W. & Gollwitzer, E. S. Neonatal mucosal immunology. *Mucosal Immunol* **10**, 5–17 (2017).

157. Thompson, M. R., Kaminski, J. J., Kurt-Jones, E. A. & Fitzgerald, K. A. Pattern recognition receptors and the innate immune response to viral infection. *Viruses* **3**, 920–940 (2011).

158. Nørregaard, R., Kwon, T.-H. & Frøkiær, J. Physiology and pathophysiology of cyclooxygenase-2 and prostaglandin E2 in the kidney. *Kidney Res Clin Pract* **34**, 194–200 (2015).

159. Kawabata, A. Prostaglandin E2 and pain-an update. *Biological and Pharmaceutical Bulletin* **34**, 1170–1173 (2011).

160. O'Banion, M. K. Prostaglandin E2 synthases in neurologic homeostasis and disease. *Prostaglandins and Other Lipid Mediators* **91**, 113–117 (2010).

161. Suzuki, J. *et al.* Roles of Prostaglandin E2 in Cardiovascular Diseases Focus on the Potential Use of a Novel Selective EP4 Receptor Agonist. *International Heart Journal* **52**, 266–269 (2011).

162. Kalinski, P. Regulation of immune responses by prostaglandin E2. *The Journal of Immunology* **188**, 21–28 (2012).

163. Hata, A. N. & Breyer, R. M. Pharmacology and signaling of prostaglandin receptors: multiple roles in inflammation and immune modulation. *Pharmacology & therapeutics* **103**, 147–166 (2004).

164. Rodríguez, M. *et al.* Polarization of the innate immune response by prostaglandin E2: a puzzle of receptors and signals. *Molecular pharmacology* **85**, 187–197 (2014).

165. Murphey, L. J. *et al.* Quantification of the major urinary metabolite of PGE2 by a liquid chromatographic/mass spectrometric assay: determination of cyclooxygenase-specific PGE2 synthesis in healthy humans and those with lung cancer. *Analytical biochemistry* **334**, 266–275 (2004).

166. Elena, S. F. & Sanjuán, R. Adaptive value of high mutation rates of RNA viruses: separating causes from consequences. *Journal of virology* **79**, 11555–11558 (2005).

167. Petrova, V. N. & Russell, C. A. The evolution of seasonal influenza viruses. *Nat Rev Microbiol* **16**, 47–60 (2018).

168. Tregoning, J. S. & Schwarze, J. Respiratory Viral Infections in Infants: Causes, Clinical Symptoms, Virology, and Immunology. *Clin Microbiol Rev* **23**, 74–98 (2010).

169. Chen, X. *et al.* Host Immune Response to Influenza A Virus Infection. *Front Immunol* **9**, 320 (2018).

170. Bossert, B. & Conzelmann, K.-K. Respiratory Syncytial Virus (RSV) Nonstructural (NS) Proteins as Host Range Determinants: a Chimeric Bovine RSV with NS Genes from Human RSV Is Attenuated in Interferon-Competent Bovine Cells. *J Virol* **76**, 4287–4293 (2002).

171. Hayman, A. *et al.* NS1 Proteins of Avian Influenza A Viruses Can Act as Antagonists of the Human Alpha/Beta Interferon Response ▿ . *J Virol* **81**, 2318–2327 (2007).

172. Hayman, A. *et al.* Variation in the ability of human influenza A viruses to induce and inhibit the IFN-β pathway. *Virology* **347**, 52–64 (2006).

173. Bartz, H., Büning-Pfaue, F., Türkel, O. & Schauer, U. Respiratory syncytial virus induces prostaglandin E2, IL-10 and IL-11 generation in antigen presenting cells. *Clin Exp Immunol* **129**, 438–445 (2002).

174. Murai, H. *et al.* IL-10 and RANTES are Elevated in Nasopharyngeal Secretions of Children with Respiratory Syncytial Virus Infection. *Allergol Int* **56**, 157–163 (2007).

175. Russell, C. D., Unger, S. A., Walton, M. & Schwarze, J. The Human Immune Response to Respiratory Syncytial Virus Infection. *Clin Microbiol Rev* **30**, 481–502 (2017).

176. Fodha, I. *et al.* Respiratory syncytial virus infections in hospitalized infants: association between viral load, virus subgroup, and disease severity. *Journal of Medical Virology* **79**, 1951–1958 (2007).

177. Launes, C. *et al.* Viral load at diagnosis and influenza A H1N1 (2009) disease severity in children. *Influenza Other Resp* **6**, e89–e92 (2012).

178. Bosis, S. *et al.* Association between high nasopharyngeal viral load and disease severity in children with human metapneumovirus infection. *J Clin Virol* **42**, 286–290 (2008).

179. Chandwani, S., Borkowsky, W., Krasinski, K., Lawrence, R. & Welliver, R. Respiratory syncytial virus infection in human immunodeficiency virus-infected children. *J Pediatrics* **117**, 251–254 (1990).

180. Shaman, J., Pitzer, V. E., Viboud, C., Grenfell, B. T. & Lipsitch, M. Absolute Humidity and the Seasonal Onset of Influenza in the Continental United States. *Plos Biol* **8**, e1000316 (2010).

181. Nguyen, J. L., Schwartz, J. & Dockery, D. W. The relationship between indoor and outdoor temperature, apparent temperature, relative humidity, and absolute humidity. *Indoor Air* **24**, 103–112 (2014).

182. Moriyama, M., Hugentobler, W. J. & Iwasaki, A. Seasonality of Respiratory Viral Infections. *Ann Rev Virol* **7**, 1–19 (2020).

183. Barbet, J. P., Chauveau, M., Labbe, S. & Lockhart, A. Breathing dry air causes acute epithelial damage and inflammation of the guinea pig trachea. *J Appl Physiol* **64**, 1851–1857 (1988).

184. Even-Tzur, N. *et al.* Climate chamber for environmentally controlled laboratory airflow experiments. *Technol Health Care* **18**, 157–163 (2010).

185. Bustamante-Marin, X. M. & Ostrowski, L. E. Cilia and Mucociliary Clearance. *Csh Perspect Biol* **9**, a028241 (2017).

186. Foxman, E. F. *et al.* Temperature-dependent innate defense against the common cold virus limits viral replication at warm temperature in mouse airway cells. *Proc National Acad Sci* **112**, 827–832 (2015).

187. Abu-Amer, Y. & Bar-Shavit, Z. Impaired Bone Marrow-Derived Macrophage Differentiation in Vitamin D Deficiency. *Cell Immunol* **151**, 356–368 (1993).

188. Pitzer, V. E. *et al.* Environmental drivers of the spatiotemporal dynamics of respiratory syncytial virus in the United States. *PLOS Pathogens* **11**, (2015).

189. Hogan, A. B., Glass, K., Moore, H. C. & Anderssen, R. S. Age Structures in Mathematical Models for Infectious Diseases, with a Case Study of Respiratory Syncytial Virus. in vol. 11 105–116 (Springer Japan, 2015).

190. Hogan, A. B., Glass, K., Moore, H. C. & Anderssen, R. S. Exploring the dynamics of respiratory syncytial virus (RSV) transmission in children. *Theoretical population biology* **110**, 78–85 (2016).

191. Moore, H. C., Jacoby, P., Hogan, A. B., Blyth, C. C. & Mercer, G. N. Modelling the seasonal epidemics of respiratory syncytial virus in young children. *PloS one* **9**, e100422 (2014).

192. Rupani, P. F. *et al.* Coronavirus pandemic (COVID-19) and its natural environmental impacts. *Int J Environ Sci Te* **17**, 4655–4666 (2020).

193. Azuma, K. *et al.* Environmental factors involved in SARS-CoV-2 transmission: effect and role of indoor environmental quality in the strategy for COVID-19 infection control. *Environ Health Prev* **25**, 66 (2020).

194. Huff, H. V. & Singh, A. Asymptomatic Transmission During the Coronavirus Disease 2019 Pandemic and Implications for Public Health Strategies. *Clin Infect Dis* **71**, 2752–2756 (2020).

195. Cohen, C. *et al.* Cohort profile: A Prospective Household cohort study of Influenza, Respiratory syncytial virus and other respiratory pathogens community burden and Transmission dynamics in South Africa, 2016–2018. *Influenza Other Resp* (2021) doi:10.1111/irv.12881.

196. Kombe, I. K., Munywoki, P. K., Baguelin, M., Nokes, D. J. & Medley, G. F. Model-based estimates of transmission of respiratory syncytial virus within households. *Epidemics-neth* **27**, 1–11 (2019).

197. Shinya, K. K. *et al.* Avian flu: influenza virus receptors in the human airway. *Nature* **440**, 435–436 (2006).

198. Gibb, R. *et al.* Zoonotic host diversity increases in human-dominated ecosystems. *Nature* **584**, 398–402 (2020).

199. Aberle, J. H. *et al.* Single Versus Dual Respiratory Virus Infections in Hospitalized Infants. *Pediatric Infect Dis J* **24**, 605–610 (2005).

200. Calvo, C. *et al.* Multiple simultaneous viral infections in infants with acute respiratory tract infections in Spain. *J Clin Virol* **42**, 268–272 (2008).

201. Lim, F. J., Klerk, N., Blyth, C. C., Fathima, P. & Moore, H. C. Systematic review and meta-analysis of respiratory viral coinfections in children. *Respirology* **21**, 648–655 (2016).

202. Rotzén-Östlund, M. *et al.* Children with multiple viral respiratory infections are older than those with single viruses. *Acta Paediatr* **103**, 100–104 (2014).

203. Achten, N. B. *et al.* Interference Between Respiratory Syncytial Virus and Human Rhinovirus Infection in Infancy. *The Journal of infectious diseases* **215**, 1102–1106 (2017).

204. Karppinen, S., Toivonen, L., Schuez-Havupalo, L., Waris, M. & Peltola, V. Interference between respiratory syncytial virus and rhinovirus in respiratory tract infections in children. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* **22**, 208.e1-208.e6 (2016).

205. Zheng, X., Song, Z., Li, Y., Zhang, J. & Wang, X.-L. Possible interference between seasonal epidemics of influenza and other respiratory viruses in Hong Kong, 2014– 2017. 1–7 (2017) doi:10.1186/s12879-017-2888-5.

206. Ånestad, G. & Nordbø, S. A. Virus interference. Did rhinoviruses activity hamper the progress of the 2009 influenza A (H1N1) pandemic in Norway? *Medical Hypotheses* **77**, 1132–1134 (2011).

207. Chan, K. F. *et al.* Investigating Viral Interference Between Influenza A Virus and Human Respiratory Syncytial Virus in a Ferret Model of Infection. *The Journal of infectious diseases* **218**, 406–417 (2018).

208. Kuitunen, I. Influenza season 2020–2021 did not begin in Finland despite the looser social restrictions during the second wave of COVID-19: A nationwide register study. *J Med Virol* **93**, 5626–5629 (2021).

209. Soo, R. J. J., Chiew, C. J., Ma, S., Pung, R. & Lee, V. Decreased Influenza Incidence under COVID-19 Control Measures, Singapore - Volume 26, Number 8—August 2020 - Emerging Infectious Diseases journal - CDC. *Emerg Infect Dis* **26**, 1933–1935 (2020).

210. Agha, R. & Avner, J. R. Delayed Seasonal RSV Surge Observed During the COVID-19 Pandemic. *Pediatrics* **148**, e2021052089 (2021).

211. Sweden, P. H. A. of. RSV-rapport vecka 39, säsong 2020/2021. https://www.folkhalsomyndigheten.se/folkhalsorapportering-statistik/statistik-a-o/sjukdomsstatistik/rsv-veckorapporter/arkiv-20212022/rsv-rapport-vecka-39/ (2021).

212. Linde, A., Rotzen-Ostlund, M., Zweygberg-Wirgart, B., Rubinova, S. & Brytting, M. Does viral interference affect spread of influenza? *Euro surveillance : bulletin Européen sur les maladies transmissibles = European communicable disease bulletin* **14**, (2009).

213. Casalegno, J. *et al.* Impact of the 2009 influenza A(H1N1) pandemic wave on the pattern of hibernal respiratory virus epidemics, France, 2009. *EuroSurveillance Monthly* **15**, 1 (2010).

214. Casalegno, J. S. *et al.* Rhinoviruses delayed the circulation of the pandemic influenza A (H1N1) 2009 virus in France. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* **16**, 326–329 (2010).

215. Weber, F., Kochs, G. & Haller, O. Inverse interference: how viruses fight the interferon system. *Viral immunology* **17**, 498–515 (2004).

216. Idborg, H. *et al.* Evaluation of urinary prostaglandin E2 metabolite as a biomarker in infants with fever due to viral infection. *Prostaglandins, Leukotrienes and Essential Fatty Acids (PLEFA)* **91**, 269–275 (2014).

217. Mayer, A. K., Bartz, H., Fey, F., Schmidt, L. M. & Dalpke, A. H. Airway epithelial cells modify immune responses by inducing an anti-inflammatory microenvironment. *European Journal of Immunology* **38**, 1689–1699 (2008).

218. Vancheri, C. The lung as a privileged site for the beneficial actions of PGE2. *Trends in immunology* **25**, 40–46 (2004).

219. Coulombe, F. *et al.* Targeted prostaglandin E2 inhibition enhances antiviral immunity through induction of type I interferon and apoptosis in macrophages. *Immunity* **40**, 554–568 (2014).

220. Liu, T. T. *et al.* RSV-induced prostaglandin E2 production occurs via cPLA2 activation: role in viral replication. *Virology* **343**, 12–24 (2005).

221. Yoshimatsu, K. *et al.* Inducible prostaglandin E synthase is overexpressed in non-small cell lung cancer. *Clinical cancer research : an official journal of the American Association for Cancer Research* **7**, 2669–2674 (2001).

222. Sznajer, Y. *et al.* Airway eicosanoids in acute severe respiratory syncytial virus bronchiolitis. *The Journal of Pediatrics* **145**, 115–118 (2004).

223. Full, F. & Gack, M. U. Prostaglandin E2: the villain in the host response to influenza virus. *Immunity* **40**, 453–454 (2014).

224. Bao, X. *et al.* Airway epithelial cell response to human metapneumovirus infection. *Virology* **368**, 91–101 (2007).

225. Kristjansson, S. *et al.* Respiratory syncytial virus and other respiratory viruses during the first 3 months of life promote a local TH2-like response. *The Journal of allergy and clinical immunology* **116**, 805–811 (2005).

226. Yamashiro, Y., Shimizu, T., Oguchi, S. & Sato, M. Prostaglandins in the plasma and stool of children with rotavirus gastroenteritis. *Journal of pediatric gastroenterology and nutrition* **9**, 322–327 (1989).

227. Rossen, J. W. A., Bouma, J., Raatgeep, R. H. C., Büller, H. A. & Einerhand, A. W. C. Inhibition of cyclooxygenase activity reduces rotavirus infection at a postbinding step. *Journal of virology* **78**, 9721–9730 (2004).

228. Hofstetter, A. O., Saha, S., Siljehav, V., Jakobsson, P.-J. & Herlenius, E. The induced prostaglandin E2 pathway is a key regulator of the respiratory response to infection and hypoxia in neonates. *Proceedings of the National Academy of Sciences of the United States of America* **104**, 9894–9899 (2007).

229. Siljehav, V., Hofstetter, A. M., Leifsdottir, K. & Herlenius, E. Prostaglandin E2 Mediates Cardiorespiratory Disturbances during Infection in Neonates. *The Journal of Pediatrics* **167**, 1207–13.e3 (2015).

230. Siljehav, V., Shvarev, Y. & Herlenius, E. Il-1β and prostaglandin E2 attenuate the hypercapnic as well as the hypoxic respiratory response via prostaglandin E receptor type 3 in neonatal mice. *Journal of applied physiology (Bethesda, Md. : 1985)* **117**, 1027–1036 (2014).

231. Sale, S. M. Neonatal apnoea. *Best Pract Res Clin Anaesthesiol* **24**, 323–336 (2010).

232. Herlenius, E. E. An inflammatory pathway to apnea and autonomic dysregulation. *Audio, Transactions of the IRE Professional Group on* **178**, 449–457 (2011).

233. Leonhardt, A., Busch, C., Schweer, H. & Seyberth, H. W. Reference intervals and developmental changes in urinary prostanoid excretion in healthy newborns, infants and children. *Acta paediatrica (Oslo, Norway : 1992)* **81**, 191–196 (1992).

234. Mitchell, M. D., Lucas, A., Etches, P. C., Brunt, J. D. & Turnbull, A. C. Plasma prostaglandin levels during early neonatal life following term and pre-term delivery. *Prostaglandins* **16**, 319–326 (1978).

235. Zhang, Y. *et al.* Pediatric Renal Ontogeny and Applications in Drug Development. *J Clin Pharmacol* **59**, S9–S20 (2019).

236. Bois, D. D. & Bois, E. F. D. A formula to estimate the approximate surface area if height and weight be known. *Archives of Internal Medicine* **XVII**, 863–871 (1916).

237. Mosteller, R. D. Simplified calculation of body-surface area. *NEJM* **17**, 1098 (1987).

238. Peters, A. M., Gordon, I. & Sixt, R. Normalization of glomerular filtration rate in children: body surface area, body weight or extracellular fluid volume? *Journal of nuclear medicine : official publication, Society of Nuclear Medicine* **35**, 438–444 (1994).

239. Newman, D. J., Pugia, M. J., Lott, J. A., Wallace, J. F. & Hiar, A. M. Urinary protein and albumin excretion corrected by creatinine and specific gravity. *Clinica chimica acta; international journal of clinical chemistry* **294**, 139–155 (2000).

240. Huang, C.-C., Wang, S.-T., Chang, Y.-C., Lin, K.-P. & Wu, P.-L. Measurement of the Urinary Lactate:Creatinine Ratio for the Early Identification of Newborn Infants at Risk for Hypoxic–Ischemic Encephalopathy. *New England Journal of Medicine* **341**, 328–335 (1999).

241. Mitchell, M. D., Lucas, A., Etches, P. C., Brunt, J. D. & Turnbull, A. C. Plasma Prostaglandin Levels During Early Neonatal Life Following Term and Pre-Term Delivery. **16**, 319–326 (1978).

242. Antonucci, R., Cuzzolin, L., Arceri, A., Dessì, A. & Fanos, V. Changes in urinary PGE2 after ibuprofen treatment in preterm infants with patent ductus arteriosus. *European journal of clinical pharmacology* **65**, 223–230 (2009).

243. Graham, G. G., Davies, M. J., Day, R. O., Mohamudally, A. & Scott, K. F. The modern pharmacology of paracetamol: therapeutic actions, mechanism of action, metabolism, toxicity and recent pharmacological findings. *Inflammopharmacology* **21**, 201–232 (2013).

244. Lee, Y.-S. *et al.* Acetaminophen selectively suppresses peripheral prostaglandin E2 release and increases COX-2 gene expression in a clinical model of acute inflammation. *Pain* **129**, 279–286 (2007).

245. Hinz, B., Cheremina, O. & Brune, K. Acetaminophen (paracetamol) is a selective cyclooxygenase-2 inhibitor in man. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* **22**, 383–390 (2008).

246. Black, A. K., Greaves, M. & Hensby, C. The effect of systemic prednisolone on arachidonic acid, and prostaglandin E2 and F2 alpha levels in human cutaneous inflammation. *Brit J Clin Pharmaco* **14**, 391–394 (1982).

247. Croxtall, J. D., Hal, P. T. W. V., Choudhury, Q., Gilroy, D. W. & Flower, R. J. Different glucocorticoids vary in their genomic and non-genomic mechanism of action in A549 cells. *Brit J Pharmacol* **135**, 511–519 (2002).

248. Silvennoinen, H., Peltola, V., Vainionpää, R., Ruuskanen, O. & Heikkinen, T. Incidence of influenza-related hospitalizations in different age groups of children in Finland: a 16-year study. *The Pediatric infectious disease journal* **30**, e24-8 (2011).

249. Wang, X. *et al.* Global burden of respiratory infections associated with seasonal influenza in children under 5 years in 2018: a systematic review and modelling study. *Lancet Global Heal* **8**, e497–e510 (2020).

250. Wen, F. T., Bell, S. M., Bedford, T. & Cobey, S. Estimating Vaccine-Driven Selection in Seasonal Influenza. *Viruses* **10**, 509 (2018).

251. Mallory, R. M., Yi, T. & Ambrose, C. S. Shedding of Ann Arbor strain live attenuated influenza vaccine virus in children 6–59 months of age. *Vaccine* **29**, 4322–4327 (2011).

252. Cowling, B. J., Chang, K. H., Peiris, J. S. M., Riley, S. & Leung, G. M. Viral shedding, clinical history and transmission of influenza. *Hong Kong Med J* **19**, 19–23 (2013).

253. Teutsch, S. M. *et al.* Ten Years of National Seasonal Surveillance for Severe Complications of Influenza in Australian Children. *Pediatr Infect Dis J* **40**, 191–198 (2020).

254. Noveroske, D. B., Warren, J. L., Pitzer, V. E. & Weinberger, D. M. Local variations in the timing of RSV epidemics. *BMC Infectious Diseases* **16**, 674 (2016).

255. Hogan, A. B., Mercer, G. N., Glass, K. & Moore, H. C. Modelling the seasonality of respiratory syncytial virus in young children. *researchgate.net* (2013).

256. Hashem, M. & Hall, C. B. Respiratory syncytial virus in healthy adults: the cost of a cold. *Journal of Clinical Virology* (2003) doi:10.1016/s1386-6532(03)00047-7.

257. Reyes, M., Eriksson, M., Bennet, R., Hedlund, K.-O. & Ehrnst, A. Regular pattern of respiratory syncytial virus and rotavirus infections and relation to weather in Stockholm, 1984--1993. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* **3**, 640–646 (1997).

258. Rhedin, S., Hamrin, J., Naucler, P. & Bennet, R. Respiratory Viruses in Hospitalized Children with Influenza-Like Illness during the H1n1 2009 Pandemic in Sweden. *PloS one* (2012) doi:10.1371/journal.pone.0051491.t003.

259. Mak, G. C., Wong, A. H., Ho, W. Y. Y. & Lim, W. The impact of pandemic influenza A (H1N1) 2009 on the circulation of respiratory viruses 2009-2011. *Influenza and other respiratory viruses* **6**, e6-10 (2012).

260. Meningher, T. *et al.* Relationships between A(H1N1)pdm09 influenza infection and infections with other respiratory viruses. *Influenza and other respiratory viruses* **8**, 422–430 (2014).

261. Hamrin, J., Bennet, R., Berner, J., Rotzén-Östlund, M. & Eriksson, M. Rates and risk factors of severe respiratory syncytial virus infection in 2008-2016 compared with 1986-1998. *Acta Paediatr* (2020) doi:10.1111/apa.15575.

262. Sommer, C., Resch, B. & Simões, E. A. F. Risk factors for severe respiratory syncytial virus lower respiratory tract infection. *Open Microbiology Journal* **5**, 144–154 (2010).

263. Sweden, P. H. A. of. *Influenza in Sweden, season 2020-2021*. https://www.folkhalsomyndigheten.se/publicerat-material/publikationsarkiv/i/influenza-in-sweden-season-2020-2021/? (2021).

264. Sweden, P. H. A. of. *Senaste RSV-rapporten — v40*. https://www.folkhalsomyndigheten.se/folkhalsorapportering-statistik/statistik-a-o/sjukdomsstatistik/rsv-veckorapporter/senaste-rsv-rapporten/ (2021).

265. Yum, S., Hong, K., Sohn, S., Kim, J. & Chun, B. C. Trends in Viral Respiratory Infections During COVID-19 Pandemic, South Korea - Volume 27, Number 6—June 2021 - Emerging Infectious Diseases journal - CDC. *Emerg Infect Dis* **27**, 1685–1688 (2021).

266. Zhang, X., Huang, F., Yang, D., Peng, T. & Lu, G. Identification of miRNA-mRNA Crosstalk in Respiratory Syncytial Virus- (RSV-) Associated Pediatric Pneumonia through Integrated miRNAome and Transcriptome Analysis. *Mediat Inflamm* **2020**, 1–13 (2020).