Epidemiology of viral respiratory infections with focus on in-hospital influenza transmission

MARTINA SANSONE

Department of Infectious Diseases
Institute of Biomedicine
Sahlgrenska Academy, University of Gothenburg

UNIVERSITY OF GOTHENBURG

Gothenburg 2020

Cover Images: Viruses and DNA (www.pixabay.com)
Trees in springtime by Martina Sansone

Epidemiology of viral respiratory infections with
focus on in-hospital influenza transmission

martina.sansone@vgregion.se

ISBN 978-91-7833-836-8 (PRINT)
ISBN 978-91-7833-837-5 (PDF)
http://hdl.handle.net/2077/63619

Layout by Guðni Ólafsson
Printed by STEMA Specialtryck AB Borås
Sweden 2020

“It is our wits against their genes”

JOSHUA LEDERBERG

Molecular biologist and geneticist
Nobel Prize Laurate 1958

ABSTRACT

Epidemiology of viral respiratory infections with focus on in-hospital influenza transmission

MARTINA SANSONE

Department of Infectious Diseases
Institute of Biomedicine
Sahlgrenska Academy, University of Gothenburg, Sweden 2020

Human Rhinovirus (HRV) and influenza virus are respiratory pathogens which represent a major global disease burden. Healthcare-associated infections (HCAIs) are increasingly recognized as a public health concern, but limited data has been published on the characteristics and epidemiology of HCAI caused by respiratory viruses. The aim of this thesis was to investigate the molecular epidemiology of HRV and influenza virus with special focus on in-hospital influenza transmission. In paper I, 114 stored respiratory samples positive for HRV, collected over a four-year period, were sequenced and compared with HRV sequences identified in other parts of the world. In paper II a nosocomial outbreak involving 20 cases with influenza B virus infection were retrospectively investigated by combining clinical and epidemiological data with molecular methods. In paper III, the characteristics of 435 hospitalized adult patients with influenza A virus infection throughout an entire year were described, whereof 114/435 (26%) were classified as HCAI. Suspected in-ward transmission was investigated by combining epidemiological investigations and whole-genome-sequencing. In paper IV, a system dynamic model for healthcare-associated influenza was developed and used in order to identify factors promoting transmission as well as effective control interventions. Conclusions: HRV infections are represented by many subtypes. HRV epidemics are highly globalised, and subtypes may circulate locally for extended time periods. Influenza B may spread rapidly within an acute-care hospital, and molecular methods can be used for outbreak analysis. In-ward transmission of influenza A occurs frequently, and healthcare-associated influenza may have a severe outcome. System dynamic modelling may be a valuable tool to illustrate in-hospital transmission of influenza. Antiviral prophylaxis seemed in our model to be the most effective control measure.

Keywords: influenza, rhinovirus, infection control, hospital outbreak, nosocomial, phylogeny, polymerase chain reaction, viral transmission, whole-genome sequencing, system dynamics.

ISBN 978-91-7833-836-8 (PRINT)
ISBN 978-91-7833-837-5 (PDF)

SAMMANFATTNING PÅ SVENSKA

Denna avhandling syftar till att fördjupa kunskapen om hur smittspridning av vanliga luftvägsvirus sker, framför allt i sjukhusmiljö.

I delarbete I jämfördes retrospektivt fynd av humant rhinovirus (HRV) i 114 luftvägsprov tagna mellan 2006 - 2010 i Göteborgsregionen med rapporterade fynd av HRV från övriga delen av världen. Vi fann en stor variabilitet av subtyper och ett globalt spridningsmönster som kan vara en delförklaring till varför HRV är ett så framgångsrikt virus. I delarbete II kartlades ett sjukhusutbrott av influensa B, där en koppling i tid och rum mellan 20 patienter kompletterades med helgenomsekvensering och fylogenetisk analys av virussekvenser. Sjukhusspridning påvisades genom detaljerad granskning av nukleotidvarianter i kombination med tidpunkt för symtomdebut och epidemiologisk koppling mellan patienter. Vi fann betydande stöd för spridning av influensa även mellan patienter som inte delat rum med varandra. I delarbete III genomfördes en retrospektiv journalgenomgång av samtliga vuxna patienter som vårdats inneliggande på Sahlgrenska Universitetssjukhuset under säsongen 2016/17 med laboratorieverifierad influensa A. Vi fann att 114/435 (26%) av patienterna uppfyllde kriterier för vårdrelaterad influensa och att dessa hade en hög dödlighet inom 30 dagar. Genom släktskapsanalys undersökte vi fall provtagna inom 7 dagar från samma vårdavdelning och fann då 8 kluster med ≥3 fall och 10 par av influensasekvenser med nära släktskap talande för att smitta på sjukhusavdelningar är vanligt förekommande. I delarbete IV beskrivs en systemdynamisk modell för smittspridning av influensavirus på ett typsjukhus skapat utifrån patientflöden, patientfaktorer och virusfaktorer. Modellen användes för att simulera olika scenarier och studera relativ effekt av olika förebyggande åtgärder för spridning av influensa inom sjukhuset. Av påverkbara faktorer visade sig profylax till samvårdade patienter och vård på enkelrum enligt vår modell vara de mest effektiva åtgärderna för att minska antalet vårdrelaterade influensafall.

Sammanfattningsvis har denna avhandling ökat kunskapen om spridningsmönster för rhinovirus, visat hur smittspridning av influensa A och B kan ske i sjukhusmiljö och hur nya molekylärbiologiska tekniker kan användas för att klargöra smittvägar och detaljstudera utbrott. Systemdynamisk modellering kan användas för att illustrera och analysera komplexa system och jämföra effekter av preventiva åtgärder vars effekter är svåra att testa i praktiken.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. Sansone M, Andersson M, Brittain - Long R, Andersson LM, Olofsson S, Westin J, Lindh M.
Rhinovirus infections in western Sweden: a four-year molecular epidemiology study comparing local and globally appearing types.
Eur J Clin Microbiol Infect Dis. 2013 Jul;32(7):947-54

II. Sansone M, Wiman Å, Karlberg ML, Brytting M, Bohlin L, Andersson LM, Westin J, Nordén R.
Molecular characterization of a nosocomial outbreak of influenza B virus in an acute care hospital setting.
J Hosp Infect. 2019 Jan;101(1):30-37

III. Sansone M, Andersson M, Gustavsson L, Andersson LM, Nordén R, Westin J.
Extensive hospital in-ward clustering revealed by molecular characterization of influenza A virus infection.
Clin Infect Dis 2020. Feb 3 [Epub ahead of print]

IV. Sansone M, Holmström P, Hallberg S, Nordén R, Andersson LM, Westin J.
Antiviral prophylaxis was the most effective preventive measure identified by system dynamic modelling of healthcare-associated influenza.
In manuscript.

CONTENT

ABBREVIATIONS

ARTI/ARI/RTI	Acute respiratory tract infection/acute respiratory infection/respiratory tract infection
CDC	U.S Centers for Disease Control and Prevention
Ct	Cycle threshold
HA	Hemagglutinin
HCAI	Healthcare-associated infection
HCW	Healthcare worker
HRV	Human rhinovirus
HRV-A	Human rhinovirus type A
HRV-B	Human rhinovirus type B
HRV-C	Human rhinovirus type C
ILI	Influenza-like illness
InfA	Influenza type A
InfB	Influenza type B
LOS	Length-of-stay
NA	Neuraminidase
NPS	Nasopharyngeal sample
PCR	Polymerase chain reaction
SD	System Dynamics
SNV	Single nucleotide variant
VP1/VP2	Viral protein 1/Viral protein 2
WGS	Whole-genome sequencing

DEFINITIONS IN SHORT

Outbreak	Occurrence of more cases of a disease than would normally be expected in a specific place or group of people over a given period.
Charlson score	A comorbidity index which predicts the one-year mortality for a patient who may have a range of a total of 22 comorbid conditions. Each condition is assigned a score depending on the risk of dying associated with each one.
Aerosol transmission	Transmission by air including small particles (< 5-10µm) possible to inhale.
Attack rate	The proportion of those becoming ill after a specific exposure.
Index case	The first case noted in an outbreak.
Primary case	The first case that brings a disease into a group of people.
Epidemic curve	A graph showing the frequency of new cases of infectious diseases over time.

INTRODUCTION

Epidemiology of viral respiratory infections with focus on in-hospital influenza transmission

MARTINA SANSONE

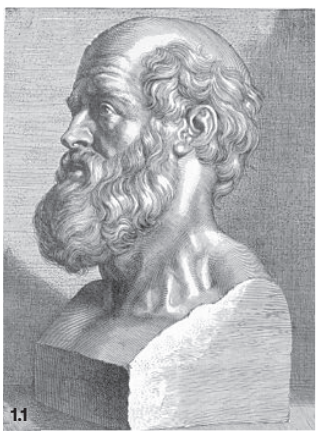
1 INTRODUCTION

Infectious diseases constituted the most serious global health issue until the beginning of the 20th century. In the history of humanity, epidemic spread of diseases like the plague, Spanish flu, or Ebola has posed significant threats to populations, in terms of both direct and indirect effects.

The role of infectious diseases may have been underestimated in the evolutionary course of human civilization, and has been considered equally important as economic and military determinants [1]. Pandemics are unpredictable and cause not only human causalities but also widespread insecurity and fear. This is being illustrated today, while the world currently gathers its forces in order to battle the pandemic spread of the newly discovered virus SARS-CoV-2.

One of the earliest reports of a highly contagious disease comes from Hippocrates, who described an influenza-like illness from northern Greece (ca. 410 B.C). The idea that some diseases are transmitted between people was developed long before the existence of microbes had been scientifically proved and formed a basis of practical infection

Figure 1: Hippocrates, Ignaz Semmelweiss and John Snow


1.1


1.2


1.3

Image source: https://commons.wikimedia.org/ Creative Commons Attribution (CC BY 2.0) license

1.1 Hippocrates by J.G de Lint Atlas van de geschiedenis der geneeskunde

1.2 Semmelweiss portrait by Agost Canzi Henry E. (1965) Große Ärzte

1.3 John Snow portrait by Thomas Johnes Barker

control. The word still used for quarantine originates from the Italian quaranta giorni, due to the 40-day isolation of ships and people practiced as a preventive measure to avoid spread of the plague in the 14th century.

Dr John Snow is considered the father of modern epidemiology, tracing a cholera outbreak to a source of contaminated water before the discovery of the infectious agent *Vibrio Cholerae*. The prevailing hypothesis at the time were transmission by foul air (often mentioned as "miasma"), a topic which interestingly have regained attention with recent reports of suspected transmission of common gastrointestinal virus by air [2, 3].

The father of infection control, Ignaz Semmelweiss, discovered that handwashing prevented the transmission of child-bed fever. Physicians however resisted his findings for several reasons. Washing hands before treating patients would be a too cumbersome procedure, involve rebuilding of hospitals and making sinks and running water available. [4]. Unfortunately, he was dismissed from his work at the hospital, and died at an insane asylum at the age of 47.

Physicians and public health specialists do not usually draw much attention from the historical record of disease control efforts. Evidence-based practices and models in the modern world instead use data removed from social contexts and expect them to be universally applicable [5].

In this thesis, the transmission patterns of HRV and influenza virus, with special focus on the hospital environment, will be discussed. Classic epidemiology will be integrated with new methods in molecular biology and computational techniques.

1.1 BACKGROUND

Respiratory tract infections (RTIs) represent the most frequent infections in humans. Adults are affected by colds approximately 2-3 times per year [6] and children up to 12 times/year [7]. Symptoms range from mild to severe, depending on factors related both to the virus itself and the host. RTIs are commonly divided into upper and lower infections. During the infection period however, different parts of the respiratory tract can be simultaneously or consecutively affected. Viral etiology is common, and a multitude of diverse viruses may cause disease. In most cases nothing but symptomatic treatment can be offered and finding a remedy for the "common cold" has been a challenge for scientists over decades. The majority of upper RTIs is caused by viruses, with a similar incidence in both low/middle and high-income countries [8].

For community-acquired pneumonia by bacterial etiology, differences in incidence rates are instead highly dependent on the country income level. Lower RTIs are the leading causes of respiratory deaths in children throughout the world and may also be caused by viruses. To underline the importance of transmission, approximately one third of all deaths from respiratory causes are due to communicable respiratory diseases [8]. However, given that respiratory viruses belong to different genera and families, have different physical properties and different viral characteristics, it is unwise and inaccurate to assume that any conclusions about one virus easily can be applied to another [9].

Even in non-epidemic situations, viral RTIs remain a major global health issue. In spite (or perhaps because) of the high prevalence, the burden of disease for viral RTIs does not gain much public attention. Human rhinovirus (HRV) and influenza virus are the two respiratory viruses with greatest

impact on the human population. Globally, HRV is the cause of >50% of common colds [10] and although HRV-related costs are likely to exceed 60 billion dollars/year, the search for a cure is still ongoing [11]. Though not typically considered a virulent pathogen, HRV also has a high potential for asthma exacerbations in children [12, 13] and worsening of chronic respiratory conditions [14].

While the success of rhinoviruses is characterized by diversity and ability to circulate all year around, the main weapon used by influenza viruses is their unique antigenic variability. This allows influenza virus to escape the immune system and cause seasonal epidemics, which every year is estimated to affect 5-10% of the world's population [15]. Contrary to rhinovirus, both vaccine and treatment options are available, although sometimes with limited effectivity.

Healthcare-associated infections (HCAIs) have increasingly being recognized as a public health concern. It has been estimated that in the European Union (EU), every year more than 91 000 deaths are attributable to the most frequent HCAIs [16]. The focus for prevention of HCAIs has been on endogenous infections or infections caused by bacteria resistant to antibiotics. Limited data are published on the characteristics and epidemiology of HCAIs caused by respiratory viruses. In the following sections, the epidemiology of HRV and transmission patterns of influenza within the hospital environment will be discussed in more detail.

THE VIRUSES

Epidemiology of viral respiratory infections with focus on in-hospital influenza transmission

MARTINA SANSONE

2 THE VIRUSES

2.1 HUMAN RHINOVIRUS

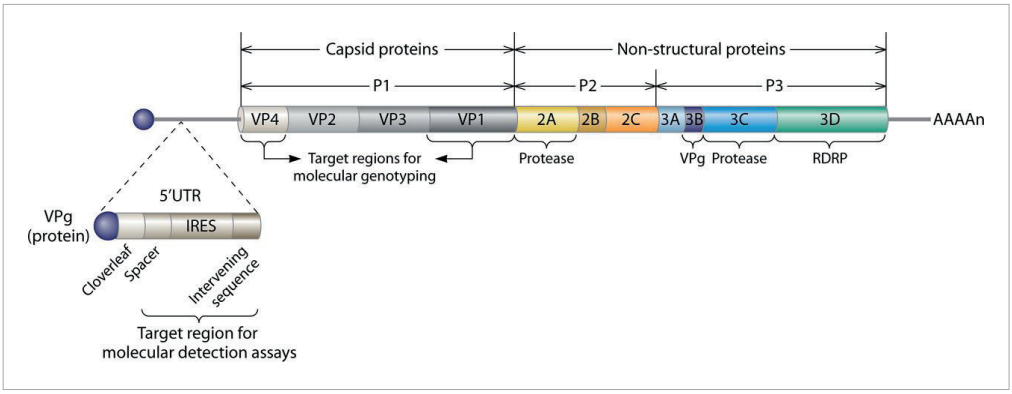
2.1.1 Basic virology

HRV are a small (around 30nm in diameter), single-stranded, non-enveloped RNA virus belonging to the family Picornaviridae, (pico-rna-virus, i.e." very small-rna-virus") and the genus Enterovirus. HRV has a genome of approximately 7.200 nucleotides which are translated into 11 proteins. Viral proteins (VP) 1-4 form the capsid, whereof VP1-3 account for the antigenic diversity of the virus (Figure 2).

Since the discovery in the 1950s, approximately 160 different subtypes have been identified and divided into three main groups, HRV-A, HRV-B and HRV-C. HRV-C uses a distinct cell-attachment mechanism and does not grow in regular cell culture [17]. There is no evidence for HRV-C being a newly emerged virus, instead the clade has probably been undetected previously. For HRV-C, type classification relies solely upon molecular techniques.

Differences in disease pathogenesis and virulence between subtypes have frequently been proposed. HRV-C, discovered as late as 2009, was initially considered to cause a more severe disease [18-20]. However well-designed studies did show that the clinical manifestations were similar between subtypes [21, 22]. To discriminate if mainly viral or host factors account for disease severity among HRV infections require further studies.

Figure 2: Genomic structure of HRV



2.1.2 Transmission

Transmission of HRVs occurs primarily by droplets or via indirect/direct contact. HRVs have been shown to survive on skin for 2 h [23], and may survive in the environment for days [24]. Because HRVs lack a lipid envelope, they are resistant to environmental perturbation as to many detergents. Use of different sanitizers, such as alcohol gels, have not been able to decrease the frequency of colds in epidemiological studies [25]. The main route of transmission has been considered to be by self-inoculation [23], however whether transmission also may occur through aerosols are not well understood.

Viral access for HRV to the respiratory tract is mainly via the nasal mucosa. In most cases the cell surface receptor ICAM-1 is used, but in some cases by the low-density-lipoprotein (LDL)-receptor. The infectious dose can depend on subtype and has not yet been determined in detail. It is likely that the infectious dose is lower than suggested by tissue culture techniques [26].

2.1.3 The disease

The incubation period is short, on average 2 days [27, 28] and duration of symptoms ranges between 7 - 14 days [7]. Clinical presentation is generally mild, and symptoms manifested in upper respiratory HRV infections are often explained by the lack of cytotoxic effects on airway epithelial cells.
Even if not cytotoxic, HRV disrupts the cell barrier function. This facilitates for bacteria to transmigrate [29], and may thereby pave the way for sinusitis, acute otitis media or other secondary bacterial infections. Lower respiratory infections such as bronchiolitis in children are a common clinical manifestation of HRV. HRV infections in young children have been identified as a non-dependent risk factor for recurrent wheezing and asthma [30].

In the adult population, influenza-like-illness (ILI) may be caused by HRV in as many as 20% of cases [31]. For immunocompromised hosts, HRV is associated with increased morbidity [32, 33]. Asymptomatic viral shedding of HRV has been reported, and HRVs are also a commonly detected co-pathogen in mixed respiratory infections. Shedding times of are relatively short (10 - 14 days) in otherwise healthy individuals [34]. In contrast, viral shedding up to 12 months has been reported in immunocompromised patients after transplantation [35, 36].

2.1.4 Epidemiology

The seasonal pattern of HRV differs from many other viral respiratory infections, as HRV infections is common all-year-round. An annual peak is noticed in early fall, possibly related to social behavior correlated with students returning to school and subsequent in-door crowding. Basic reproductive number (R0) for rhinovirus is estimated to be around 1,2-1,5 [37, 38].

2.1.5 Immunology

Immunological responses to HRV infections involve both the innate and the adaptive immune system. IL-8 has been shown to be an important factor for clinical outcome. After experimental virus inoculation, IL-8 levels in nasal lavage peak after 48-72 h and correlate with symptom severity [39]. Humoral immune responses are probably also important but not well understood. Antibodies (IgG as well as secretory IgA) are detected after 1-2 weeks of infection and may remain elevated for years [40]. The main challenge for the human immune system, and for future vaccine developers, is the high number of different serotypes with incomplete cross-protective immunity [41]. In order to find an effective strategy to battle HRV, not a single key needs to be found but a master key to open hundreds of locks.

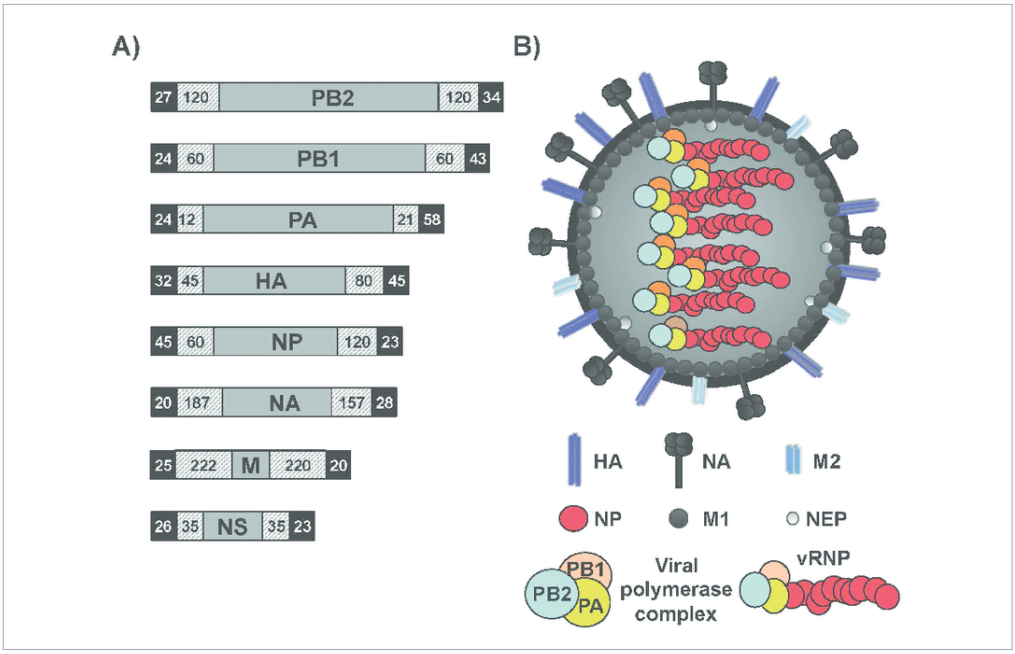
2.2 INFLUENZA VIRUS

2.2.1 Basic virology

Influenza viruses measures around 80-120 nm in diameter and is a single-stranded RNA virus belonging to the Orthomyxoviridae family. The segmented genome consists of approximately 14 000 nucleotides within a lipid envelope which translate into at least 17 proteins (Figure 3). Influenza is divided into type A, B and C [42]. While influenza A (InfA) and B (InfB) are involved in seasonal epidemics, type C (InfC) generally causes a mild disease. Influenza A was first isolated 1933 and Influenza B in 1936.

Based on antigenic properties, InfA is further classified into subtypes where the surface glycoproteins hemagglutinin (HA) and neuraminidase (NA) account for the differences. Sixteen different types of HA (H1-H16) and 9 different types of NA are described, which all may be combined to develop new InfA subtypes. For InfB there are instead two distinctly separate lineages circulating in humans, Victoria (VIC) and Yamagata (YAM), classified due to a divergence of 27 amino acids in the HA gene [43]. Being an RNA virus with high mutation rate (2.0 × 10−6 for InfA and 0.6 × 10−6 for Inf B per site/cycle) [44] and without proofreading function during replication, influenza is regarded as an unstable virus which constantly undergo changes.

Figure 3A: Genome organization and **3B:** Virion structure for influenza A.



Reprint with permission from: https://www.researchgate.net/figure/Influenza-A-virus-IAV-genome-organization-and-virion-structure-A-Genome_fig1_304397258 [accessed 31 March 2020]

2.2.2 Transmission

InfA is a zoonosis with birds as the natural host. Only subtypes H1-H3 and N1-N2 have been involved in transmission between human subjects. Avian influenza occasionally spread from birds to humans and may cause severe disease with high mortality, but none of the various types of "bird flu" have yet reached an epidemic stage although suspected human-to-human transmission has been reported [45, 46].

Differences in disease outcome and clinical picture have been suggested to be related to level of exposure and mode of transmission [47-49]. Aerosolized influenza viruses are infectious at a dose much lower than by nasal instillation [50]. Intranasally administered influenza virus uncommonly causes lower respiratory tract infections in experimentally infected volunteers [51]. Indirect contact is also regarded as a relevant mode of transmission. Influenza viruses may last at steel surfaces for up to 24 h, but rapidly decreases on hands by 15 min [52-55].

Accumulated point mutations in the HA and NA gene cause minor changes in surface antigens, which combined with selective pressure result in what is known as antigenic drift. This mechanism occurs in all three types and is a key factor to successively escape the immune system. Antigenic shift on the contrary, is a sporadic event occurring at irregular intervals and which only includes InfA. It is based on a reassortment of genes and results in a novel virus strain. It may transmit directly from birds to humans but more likely occurs through an exchange of genes within an intermediate host simultaneously infected by both avian and human influenza, such as pigs [56]. Antigenic shift has a more dramatic impact on global health and a potential of pandemic spread because of the low prevalence of protective antibodies in the population. Severity may not generally be greater, but due to the large number of persons infected, the total amount of severe infections will be high.

2.2.3 The disease

The clinical presentation of influenza is characterized by a sudden onset (in German illustratively called 'blitzkatarr') of systemic reactions including fever, chills, myalgia combined with symptoms of RTI such as dry cough, nasal discharge and sore throat (Figure 5). The incubation period is short, 24-48 h, with a median of 1.4 days for InfA and 0.6 days for InfB [57]. Fever may rise as high as 40-41 ^{o}C in the first days of illness [58] and typically lasts around 3 - 8 days. The clinical symptoms of InfB infections are generally similar to those of InfA [59]. Historically, the diagnosis of influenza (or ILI) has been based upon clinical presentation, not easily distinguished from other RTIs. High fever may affect the cardiovascular system and inflammatory engagement of bronchioli can block the flow of oxygen and gas exchange in the lungs. Infection of alveolar epithelial cells appears to trigger acute respiratory distress syndrome (ARDS) [60].

Influenza infections are further are associated with primary viral pneumonia, bronchiolitis and croup [58, 61, 62]. Secondary bacterial pneumonia is a well-known and potentially severe complication. In the 1918, 1957 and 2009 pandemics, a large proportion of the fatalities was associated with bacterial pneumonia [63, 64]. Influenza may also affect other organs and cause myocarditis, encephalitis as well as exacerbations of underlying heart diseases [65]. Chow et al recently reported a high frequency (47%) of non-respiratory diagnoses in a large study including almost 90 000 hospitalized adults with laboratory confirmed influenza [66]. In this report, 5.1% had a non-respiratory diagnosis only, of which sepsis was the most common.

It has been hypothesized that severity differs across types and subtypes. Thompson et al found the highest number of hospitalizations and influenza-associated deaths during seasons in which H3N2 was the dominant subtype, followed by seasons dominated by InfB or H1N1 [67]. This was later confirmed in other studies [67-69] and also by the Public Health Agency of Sweden [70]. Nevertheless, it has been difficult to identify strain-specific determinants of severity due to multiple confounders such as diversity in study populations, settings and influenza case definitions [71]. The comparatively higher burden of disease associated with H3N2 may be due to the greater susceptibility to this subtype in the elderly, as these represent the largest group at risk for severe influenza [72]. Patients hospitalized for influenza with acute non-respiratory diagnoses have been reported to have a significantly higher frequency of underlying medical comorbidities compared with patients with respiratory diagnoses.

Stratifying risks is important for strategic planning of influenza management. The influenza-attributable mortality has been assessed with heterogenous results in numerous studies as both host, pathogen, setting and methodological factors need to be considered [73]. WHO estimated that influenza is associated with 290 000 to 650 000 deaths from respiratory causes alone [74]. Increased risk for severe influenza infections among adults with specific chronic medical conditions were recently reported and compared with those without such conditions. The largest risks occurred with congestive heart failure, end-stage renal disease, coronary artery disease and chronic obstructive pulmonary disease [75]. Hospitalization rates are high among the 'elderly elderly'. For adults aged 75-84 years and ≥85 years rates were reported to be 1.4-3.0 and 2.2-6.4 times greater respectively, than rates for adults aged 65-74 years [76]. In Sweden, the Public Health Agency reported a 30-day mortality rate among confirmed cases between 2.9-5.6% season 2015-2019, whereof in season 2018/19, 86% were >65 years old [77].

2.2.4 Immunology

In order to enter the human cell, HA binds to sialyloligosaccharide receptors at the surface of the hosts cells, while NA enables release of viral particles by enzymatic cleavage. as The adaptive immune memory is highly strain specific, why previous influenza exposure have an impact on future susceptibility. The first influenza type a child is exposed to has a profound effect on immunity [78]. This has been proposed as a reason why the burden of mortality for the H1N1 pandemic in 2009-10 was shifted towards patients younger than 65 years of age, since the elderly were more likely to previously have encountered related subtypes [79].

2.2.5 Epidemiology

The impact of influenza can be described in terms of transmissibility estimated by effective reproduction number (R1). The median R1 value for the 2009 pandemic was 1.46 for the first wave and 1.48 for the second wave. The median R1 value for seasonal influenza was 1.28 according to a systematic review by Biggerstaff et al in 2014 [80].

The seasonal pattern of influenza is well known but much less understood. There is a gap in how studies combine immunology, mathematics, epidemiology and virology to form a picture of flu seasonality [81]. In temperate climate, the epidemic on-set is generally seen in December, and lasts for approximately 6–12 (in median 10) weeks [82]. Increased transmission during cold weather has been related to both indoor crowding and facilitated

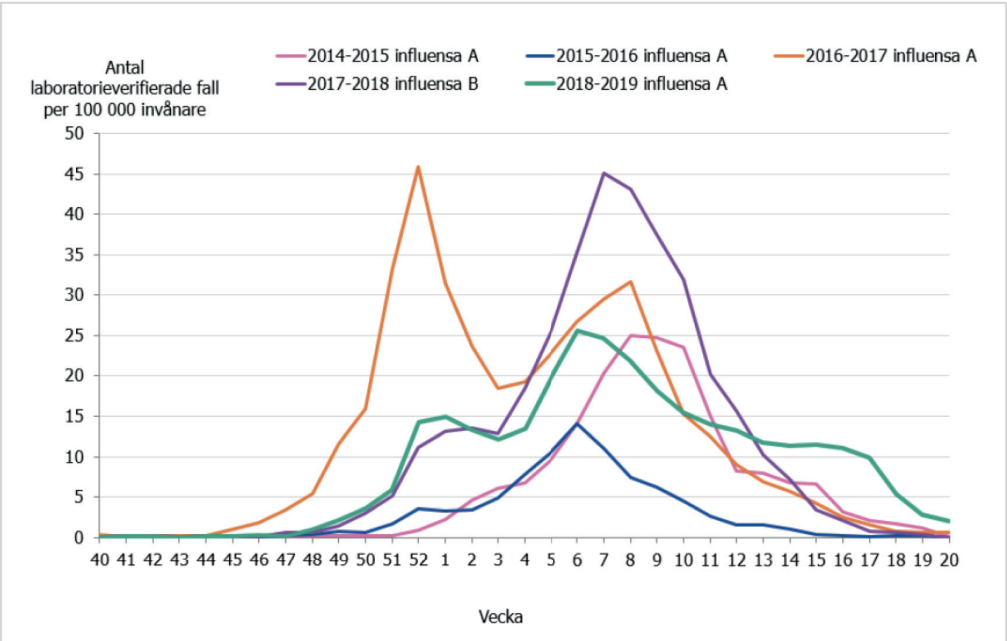
spread in dry air [83-85]. Epidemics are less pronounced in the tropics/subtropics, but the incidence in these areas is higher during humid and rainy conditions [86].

Annual influenza epidemics typically affect 5-10% of the adult population [15]. Influenza surveillance aim to detect the start and duration as well as to monitor trends during the influenza season. In Sweden, the Public Health Agency publish weekly reports and provide key data and analysis (Figure 4). Globally coordinated epidemiologic and virologic surveillance are essential. For Europe ECDC (European Center for Disease Control and Prevention) report to WHO's Global Influenza Surveillance and Response System (GISRS).

Figure 4: Total number of laboratory-confirmed cases of influenza per week and season.

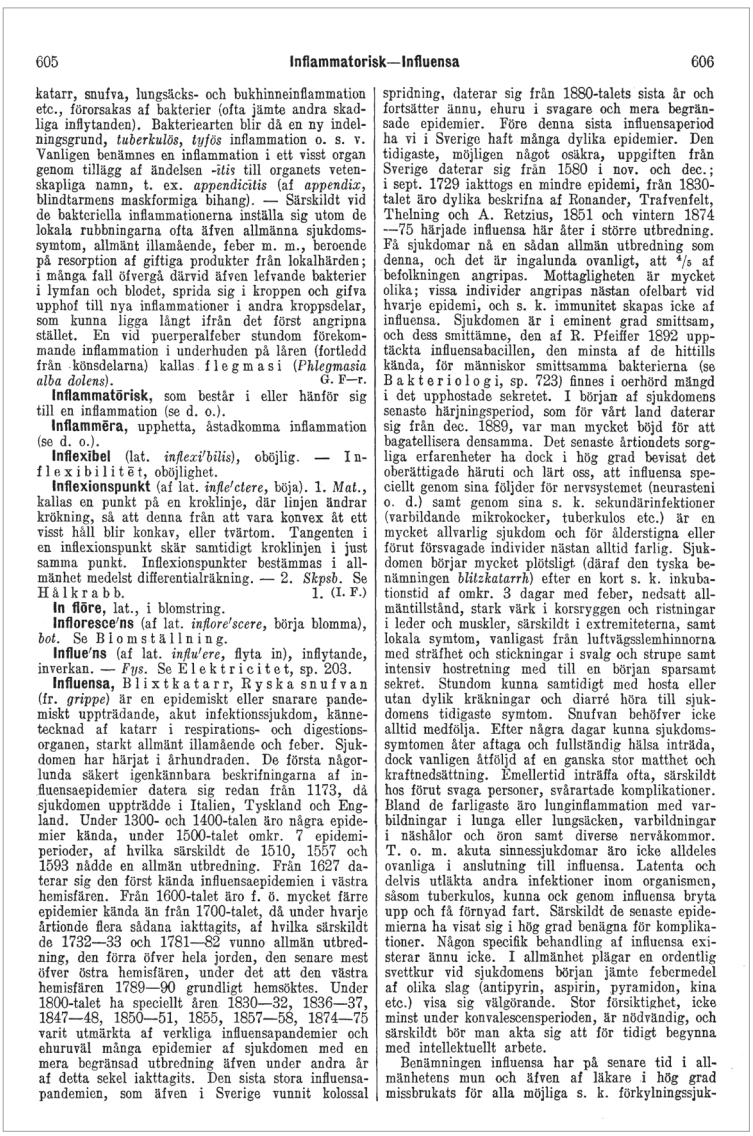
2.2.6 Prevention and treatment

The most effective method for controlling influenza is undoubtedly vaccination [87, 88]. WHO is responsible for recommendations regarding seasonal composition [89], which normally contain antigens from InfA (H3N2 and H1N1) as well as either one or two circulating InfB strains (tri or quadrivalent vaccines). Evaluation of vaccines is made either in aspect of efficacy or effectiveness. Whilst vaccine efficacy refers to randomized control studies measuring specific reduction in rates of laboratory confirmed infection, effectiveness is determined by observational data. Well-matched vaccines usually report the effectiveness to be around 50-60% in healthy adults [90]. Most countries recommend vaccination for defined risk groups and healthcare workers. Despite strong recommendations, immunization rates remain around 50% in Sweden among elderly >65 years (well below the 75% goal set by WHO) and coverage in other risk groups is low, in Sweden estimated to be only ~2% [91].



Antiviral treatment options for influenza are currently dominated by neuraminidase inhibitors, where oseltamivir is the most extensively used drug of choice. Nevertheless, data regarding effectiveness of neuraminidase inhibitors are variable and highly dependent on administration early in the disease course, preferably within 48 hours of onset [92]. Side-effects are generally mild (mainly gastrointestinal such as nausea) and resistance is uncommon [93]. In randomized control trials, duration of clinical symptoms was shortened by approximately 1 day by oseltamivir [94]. The use of preventive treatment in infection control will be further discussed in section 7.9.

Figure 5: Description of Influenza from Nordic Family book, 1910.



605 **Inflammatorisk—Influensa** 606

katarr, snufva, lungsäcks- och bukhinneinflammation etc., förorsakas af bakterier (ofta jämte andra skadliga inflytanden). Bakteriearten blir då en ny indelningsgrund, *tuberkulös*, *tyfös* inflammation o. s. v. Vanligen benämnes en inflammation i ett visst organ genom tillägg af ändelsen *-itis* till organets vetenskapliga namn, t. ex. *appendicitis* (af *appendix*, blindtarmens maskformiga bihang). — Särskildt vid de bakteriella inflammationerna inställa sig utom de lokala rubbningarna ofta äfven allmänna sjukdomssymtom, allmänt illamående, feber m. m., beroende på resorption af giftiga produkter från lokalhärden; i många fall öfvergå därvid äfven lefvande bakterier i lymfan och blodet, sprida sig i kroppen och gifva upphof till nya inflammationer i andra kroppsdelar, som kunna ligga långt ifrån det först angripna stället. En vid puerperalfeber stundom förekommande inflammation i underhuden på låren (fortledd från könsdelarna) kallas flegmasi (*Phlegmasia alba dolens*). G. F—r.

Inflammatörisk, som består i eller hänför sig till en inflammation (se d. o.).

Inflammēra, upphetta, åstadkomma inflammation (se d. o.).

Inflexibel (lat. *inflexi'bilis*), oböjlig. — Inflexibilitēt, oböjlighet.

Inflexionspunkt (af lat. *infle'ctere*, böja). 1. *Mat.*, kallas en punkt på en kroklinje, där linjen ändrar krökning, så att denna från att vara konvex åt ett visst håll blir konkav, eller tvärtom. Tangenten i en inflexionspunkt skär samtidigt kroklinjen i just samma punkt. Inflexionspunkter bestämmas i allmänhet medelst differentialräkning. — 2. *Skpsb.* Se Hålkrabb. 1. (I. F.)

In flōre, lat., i blomstring.

Inflorescē'ns (af lat. *inflore'scere*, börja blomma), *bot.* Se Blomställning.

Influe'ns (af lat. *influ'ere*, flyta in), inflytande, inverkan. — *Fys.* Se Elektricitet, sp. 203.

Influensa, Blixtkatarr, Ryska snufvan (fr. *grippe*) är en epidemiskt eller snarare pandemiskt uppträdande, akut infektionssjukdom, kännetecknad af katarr i respirations- och digestionsorganen, starkt allmänt illamående och feber. Sjukdomen har härjat i århundraden. De första någorlunda säkert igenkännbara beskrifningarna af influensaepidemier datera sig redan från 1173, då sjukdomen uppträdde i Italien, Tyskland och England. Under 1300- och 1400-talen äro några epidemier kända, under 1500-talet omkr. 7 epidemiperioder, af hvilka särskildt de 1510, 1557 och 1593 nådde en allmän utbredning. Från 1627 dateras sig den först kända influensaepidemien i västra hemisfären. Från 1600-talet äro f. ö. mycket färre epidemier kända än från 1700-talet, då under hvarje årtionde flera sådana iakttagits, af hvilka särskildt de 1732—33 och 1781—82 vunno allmän utbredning, den förra öfver hela jorden, den senare mest öfver östra hemisfären, under det att den västra hemisfären 1789—90 grundligt hemsöktes. Under 1800-talet ha speciellt åren 1830—32, 1836—37, 1847—48, 1850—51, 1855, 1857—58, 1874—75 varit utmärkta af verkliga influensapandemier och ehuruväl många epidemier af sjukdomen med en mera begränsad utbredning äfven under andra år af detta sekel iakttagits. Den sista stora influensapandemien, som äfven i Sverige vunnit kolossal spridning, daterar sig från 1880-talets sista år och fortsätter ännu, ehuru i svagare och mera begränsade epidemier. Före denna sista influensaperiod ha vi i Sverige haft många dylika epidemier. Den tidigaste, möjligen något osäkra, uppgiften från Sverige daterar sig från 1580 i nov. och dec.; i sept. 1729 iakttogs en mindre epidemi, från 1830-talet äro dylika beskrifna af Ronander, Trafvenfelt, Thelning och A. Retzius, 1851 och vintern 1874—75 härjade influensa här åter i större utbredning. Få sjukdomar nå en sådan allmän utbredning som denna, och det är ingalunda ovanligt, att $^4/_5$ af befolkningen angripas. Mottagligheten är mycket olika; vissa individer angripas nästan ofelbart vid hvarje epidemi, och s. k. immunitet skapas icke af influensa. Sjukdomen är i eminent grad smittsam, och dess smittämne, den af R. Pfeiffer 1892 upptäckta influensabacillen, den minsta af de hittills kända, för människor smittsamma bakterierna (se Bakteriologi, sp. 723) finnes i oerhörd mängd i det upphostade sekretet. I början af sjukdomens senaste härjningsperiod, som för vårt land daterar sig från dec. 1889, var man mycket böjd för att bagatellisera densamma. Det senaste årtiondets sorgliga erfarenheter ha dock i hög grad bevisat det oberättigade häruti och lärt oss, att influensa speciellt genom sina följder för nervsystemet (neurasteni o. d.) samt genom sina s. k. sekundärinfektioner (varbildande mikrokocker, tuberkulos etc.) är en mycket allvarlig sjukdom och för ålderstigna eller förut försvagade individer nästan alltid farlig. Sjukdomen börjar mycket plötsligt (däraf den tyska benämningen *blitzkatarrh*) efter en kort s. k. inkubationstid af omkr. 3 dagar med feber, nedsatt allmäntillstånd, stark värk i korsryggen och ristningar i leder och muskler, särskildt i extremiteterna, samt lokala symtom, vanligast från luftvägsslemhinnorna med sträfhet och stickningar i svalg och strupe samt intensiv hostretning med till en början sparsamt sekret. Stundom kunna samtidigt med hosta eller utan dylik kräkningar och diarré höra till sjukdomens tidigaste symtom. Snufvan behöfver icke alltid medfölja. Efter några dagar kunna sjukdomssymtomen åter aftaga och fullständig hälsa inträda, dock vanligen åtföljd af en ganska stor matthet och kraftnedsättning. Emellertid inträffa ofta, särskildt hos förut svaga personer, svårartade komplikationer. Bland de farligaste äro lunginflammation med varbildningar i lunga eller lungsäcken, varbildningar i näshålor och öron samt diverse nervåkommor. T. o. m. akuta sinnessjukdomar äro icke alldeles ovanliga i anslutning till influensa. Latenta och delvis utläkta andra infektioner inom organismen, såsom tuberkulos, kunna ock genom influensa bryta upp och få förnyad fart. Särskildt de senaste epidemierna ha visat sig i hög grad benägna för komplikationer. Någon specifik behandling af influensa existerar ännu icke. I allmänhet plägar en ordentlig svettkur vid sjukdomens början jämte febermedel af olika slag (antipyrin, aspirin, pyramidon, kina etc.) visa sig välgörande. Stor försiktighet, icke minst under konvalescensperioden, är nödvändig, och särskildt bör man akta sig att för tidigt begynna med intellektuellt arbete.

Benämningen influensa har på senare tid i allmänhetens mun och äfven af läkare i hög grad missbrukats för alla möjliga s. k. förkylningssjuk-

INFECTION PREVENTION AND CONTROL

Epidemiology of viral respiratory infections with focus on in-hospital influenza transmission

MARTINA SANSONE

3 INFECTION PREVENTION AND CONTROL

3.1 GENERAL ASPECTS

Infection control units mainly focus on practical implications to reduce transmission, managing outbreaks, and performing surveillance within a wide range of communicable diseases and healthcare settings. The aim is to protect patients and HCWs by breaking the chain of infection, a goal which can be perceived as indirect and diffuse for those working in close contact with patients. Ethical considerations are common, such as situations arising when a patient in need of care at the same time is considered hazardous for other patients or staff.

In the 1980s it was demonstrated that surveillance and infection control practices (including trained professionals) could prevent healthcare-associated infections [95]. In 1996 CDC introduced guidelines for standard precautions, which now are widely adopted [96]. These assume that all patients carry transmissible organisms, although they may be asymptomatic. Since then, the need for infection control programs has grown while medicine has become more complex and healthcare costs continues to increase. The high burden of HCAIs forces administrations around the world to try to find the best use of limited resources.

Infection control are often constituted of a bundle of measures, why the effect of single procedures for prevention is difficult to scientifically evaluate. To add more complexity, risk analysis of transmission does not only include the likelihood of transmission, but also a need for estimating the consequences of the undesired event. This is facilitated by standardizations in how to define cases and concepts within the infection control field as well as good communication skills.

3.2 HEALTHCARE-ASSOCIATED INFECTIONS

Healthcare-associated infections (HCAIs) are infections occurring in a patient during the process of care in a hospital or another healthcare facility, which was not present or incubating at the time of admission [97]. Occupational infections among HCWs are also included, but rarely reported. In EU/EEA, approximately 4 131 000 patients are affected by 4 544 100 episodes of HCAIs every year. HCAIs further account for 16 million extra days of hospital stay and 37 000 attributable deaths annually, but also contribute to additional 110 000 deaths. The economic burden (in direct costs only) is estimated to approximately € 7 billion per year [98]. It remains unclear what the most effective strategy is to improve adherence to standard precautions [99].

The definition of HCAIs rely upon time limits, where onset of symptoms >48 h after admission or <48 h after a previous discharge is the most common [100] HCAI has to some extent replaced the

terms nosocomial or hospital-infection. However, it does not include matters of known exposure/ epidemiologic links and is not equal to the more specific term 'hospital-acquired infection'.

The lack of knowledge regarding HCAIs caused by respiratory viruses may partly be explained by the difficulties in surveillance. Viral RTIs are rarely notifiable diseases and contact tracing is seldom feasible, nor relevant. Healthcare-associated infections of viral respiratory origin are in many aspects different as to those of bacterial origin. Bacteria are responsible for important HCAIs such as central-line-associated bloodstream infections, ventilator-associated pneumonia or catheter-associated urinary tract infections [101], but viral HCAIs need to be addressed in a different manner. Asymptomatic carriage of respiratory viral infections is rare why screening of patients in the way it is performed for bacteria is not possible. Indirect transmission through contaminated surfaces is less important for viruses compared with bacteria, which may survive on surfaces and remain infectious for long time periods (e.g. vancomycin-resistant enterococci and MRSA) [102, 103].

Figure 6: Public advice from the Ministry of Health, Great Britain during World War II.

Poster designed by British cartoonist HM Bateman.

LABORATORY METHODS

Epidemiology of viral respiratory infections with focus on in-hospital influenza transmission

MARTINA SANSONE

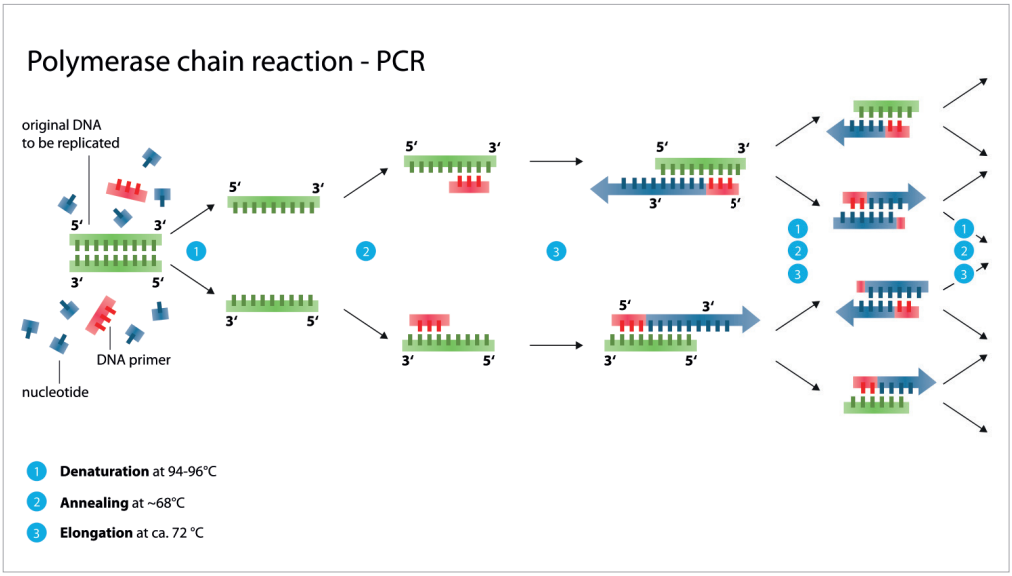
4 LABORATORY METHODS

4.1 POLYMERASE CHAIN REACTION (PCR)

The PCR method was first described in 1983 and has since then revolutionized diagnostic virology. The process is described in Figure 7. Different nucleic acid amplifications tests are now the standard method to detect virus in various types of biologic samples, where so-called 'primers' are carefully selected to match conserved sequences of the targeted gene to allow identification. Development of multiplex methods (where several pathogens at the same time can be detected) and automated extractions have further enabled increased use and shortening of turnaround times.

Besides mere pathogen identification, real-time PCR (sometimes referred to as qPCR) allows for a semi-quantitative estimation of viral load in the analyzed sample. By adding specific oligonucleotides, 'probes', it is possible to follow each cycle of the PCR-process by emitted fluorescent signals, which also can be plotted as a curve. The cycle when fluorescent detection occurs is referred to as the cycle threshold (Ct) value. This value is proportional to the logarithm of the target concentration before amplification.

Figure 7. Polymerase chain reaction



Multiplex PCR refers to a process when multiple primer-sets are used within the same run. This has been beneficial in reducing workload and cost, in addition to assist the treating physician in finding the correct diagnosis amongst the multitude of pathogens causing RTI. Choosing which primers to combine for multiplexing needs precision and optimization, as some combinations does not fit well together and therefore may hamper performance below an acceptable level.

Even though PCR has added considerable value as a diagnostic tool, there are some methodological limitations and challenges. It is impossible to discriminate between viable and non-viable virus. Detection and clinically relevant infection are two different things. Cross-contamination may lead to false positive results. Multiplex analyzes may detect several pathogens which can lead to difficulties in result interpretation. Primers may attach to sequences similar to the target gene. And finally, the continuous evolution of virus can be a challenge. Mismatch of primers may occur if the targeted genes undergo changes, paving the way for emerging viruses to spread undisturbedly without detection.

4.2 SEQUENCING

After the discovery of DNA by Watson and Crick in the 1950s, techniques to 'read' the genome by determining the order of nucleotides in biological samples was developed over several years. Since then, a rapid evolvement has occurred, in which sequencing minor fragments of single genes has moved to a widespread availability of whole-genome-sequencing (WGS).

Fredrick Sanger developed a technique based on the detection of radiolabeled fragments after a two-dimensional fractioning [104]. This allowed for the birth of 'first-generation' DNA sequencing, where fragments are broken at specific bases and then runned on a polyacrylamide gel. Thus, the position of specific nucleotides can be determined. A breakthrough for sequencing technology came in 1977 with the use of deoxyribonucleotide analogues. By mixing radiolabeled nucleotides into a DNA extension reaction, fragments of each possible length can be produced and then illustrated as radioactive bands at a corresponding position on the gel. After several improvements, the so-called 'Sanger sequencing' became the most common sequencing technique for years to come.

Concurrent development of PCR provided means of generating the high concentrations of DNA which are required for sequencing. In 'second generation' sequencing, machines allowed for mass parallelization of reactions, which greatly increased the amount of DNA possible to sequence in one run [105]. After parallelization, bridge amplification techniques followed, where replicating DNA strands are used to prime the next round of polymerization. The DNA molecules are then passed over a lawn of complementary oligonucleotides bound to a flow-cell, after which subsequent PCR produces neighboring clusters from each individual flow-cell [106].

Due to remarkable progress in technology in the last decade, several sequencing companies with different methodologies have appeared. One of the most important perhaps being Illumina [107] and Ion Torrent which use the first so-called 'post-light' sequencing, involving neither fluorescence nor luminescence technology [108]. The genomic revolution can be illustrated by a doubling of sequencing capability which occurred every 5 months between 2004 and 2010 [109]. After providing a great amount of information in terms of sequences of various

length ('reads') and number ('depth'), a process of mapping the reads to reference sequences need to follow. This led scientists in the field of molecular biology to move in front of computers instead of doing classical laboratory work.

We have now entered the 'third-generation' sequencing era, with possibilities of massive reading of DNA fragments at the length of hundreds of base pairs, and the stored amount of sequence data is growing continuously. Nanopore sequencing can produce ultra-long read length at a high speed. In 2014, the platform MinION was released [110] which is a handheld 90 g device that can plug into any computer with a standard USB port. This allows for portable sequencing in the field with less high-skilled training required. For example, in Guinea Ebola viruses were sequenced two days after sample collection [111]. Sequencing has even been performed in remote field locations such as the dry valleys of Antarctica [112].

For influenza surveillance, public health laboratories have previously relied upon Sanger sequencing of the HA gene, with focus on the dominant virus lineage within an infected individual, the so-called 'consensus sequence'.

The detailed information obtained by WGS however provides opportunities to closely monitor the genetic profiles of circulating influenza strains. This may be a useful contribution in order to detect emerging strains, antiviral drug mutations and optimize vaccine selection [113] and is illustrated by recent reports on influenza surveillance based on WGS [114, 115]. How to put extensive molecular data into practical use lies ahead of us. Future development will probably shift to be driven by applications instead of technological advances.

4.3 PHYLOGENETICS

Phylogeny is a way to classify organisms and organize genetic information where the relationships are given by the degree and kind of evolutionary distance. Traditionally it has been based upon morphology, but since the birth of molecular phylogeny in 1962 [116], genetic sequence data forms the basis for phylogenetic studies and molecular epidemiology.

The genetic relationship between species is commonly illustrated by a phylogenetic tree, which is a graphical representation that ideally has a root, nodes and branches of different lengths. A root is often referred to as being the last common ancestor. Division into clades is based upon the idea that members of one group share a common evolutionary history and are more closely related to each other than to members of any other group.

As previously described, molecular sequence data has the recent years become increasingly available. In addition, refined computer algorithms for tree construction have been developed. Methods for phylogenetic tree construction are often being classified into two groups by the use of the maximum likelihood/maximum parsimony approach or by a distance matrix.

Maximum likelihood (ML) assigns quantitative probabilities to mutational events, rather than merely counting them. This method compares possible phylogenetic trees based on ability to predict observed data. The tree that has the highest probability of producing the observed sequences is preferred [117]. Maximum likelihood seems to be an appealing way to estimate phylogenies [118].

Maximum parsimony (MP) aims to create the phylogenetic tree which requires the least evolutionary

change. It may however suffer from long branch attraction, a problem that may lead to incorrect trees in rapidly evolving lineages [119].

Another way of measuring relatedness is by a distance matrix, which can estimate the mean number of nucleotide differences between two related sequences. It is recommended to include at least one distantly related sequence for the analysis as a sort of negative control.

In addition, phylogenetic tree construction often involves bootstrapping analyses. Bootstrapping is a way of rebuilding the tree and testing if the nodes remain unchanged through many iterations. For example, if the same node is recovered in 95 of 100 iterations of resampling, the result is a bootstrap value of 95%. This should be interpreted as the node is well supported, not that the branches have a 95% genetic similarity. Several software packages are available for tree construction, such as the highly recommended MEGA®, which also allow for a visual inspection of alignments. Ideally, for reliable data sets, including multiple correct sequence alignments, any of the methods described above would be found largely accurate.

One major concern in phylogenetic tree construction need to be addressed: the level of uncertainty with respect to the true evolutionary relationships. Both analytical and biological factors as well as known and unknown factors, may cause incongruence. Resolving phylogenetic incongruence is however not easy; a problem may become more complicated when the attempts of resolving one negative factor instead introduce a new negative factor [120].

4.4 BIOINFORMATICS

Bioinformatics is a fast-moving field with unclear boundaries, but can be perceived as a way of processing extensive data from biological systems and place it into context.

One of the most used and updated sequence databases is GenBank ®, which provides an annotated collection of all publicly available DNA sequences. The database offers various ways to search and retrieve data, for example by BLAST searches (Basic Local Alignment Search Tool), where similar regions within nucleotide or protein sequences can be found and compared with each other. The largest collection of influenza sequences is GISAID (Global Initiative on Sharing All Influenza Data) through its database Epiflu, hosted by the German government.

Currently, there is no standard for 'pipeline development' in whole genome sequencing. However, bioinformatic algorithms are nevertheless crucial tools for comparative and functional genomics, such as sequence alignment, assembly, identification of single nucleotide polyforms or variants (SNP/SNV), gene prediction, and quantitative analysis of transcription data [121]. In order to add scientific value, genomic data needs to be stored, shared, and enabled for reanalysis when new hypotheses are generated. In molecular epidemiology, web-based tools for visualizing and comparing datasets may further supply public health laboratories with important information.

Several programs are available to align reads to a reference genome or to assemble them de novo [122], but may differ in aspects such as type of sequencing platform, read length, expected genome size, length of longest repetitive elements, and whether paired-end reads are in use. Interdisciplinary to its nature, bioinformatics combines biology, computer science, information engineering, mathematics and statistics.

AIMS

Epidemiology of viral respiratory infections with focus on in-hospital influenza transmission

MARTINA SANSONE

5 AIMS

The overall aim of this thesis was to investigate the transmission patterns of rhinovirus and influenza virus infections, especially within the hospital environment and more specifically to:

- Describe the seasonal pattern of HRV types over four consecutive seasons in one geographic region (Paper I)
- Investigate a hospital outbreak of influenza B by combining clinical and epidemiological data with molecular methods (Paper II)
- Describe the characteristics of patients with influenza A virus infection at a large acute-care hospital across an entire season and to use whole-genome sequencing to investigate in-ward transmission (Paper III)
- Develop a system dynamic model to illustrate healthcare-associated influenza transmission and to use the model to identify effective control interventions (Paper IV)

METHODS

Epidemiology of viral respiratory infections with focus on in-hospital influenza transmission

MARTINA SANSONE

6 METHODS

6.1 SETTINGS

Data included in this thesis were collected retrospectively from Region Västra Götaland 2006-2010 (Paper I), more specifically from Kungälv hospital 2016 (Paper II) and Sahlgrenska University Hospital between 2016-2019 (Paper III-IV). Sahlgrenska University hospital is a teaching facility with ~1900 beds including three main emergency departments (ED) for adult patients and Kungälv hospital is a medium sized hospital with ~200 beds and one ED.

6.2 DIAGNOSTIC MULTIPLEX REAL-TIME PCR FOR RESPIRATORY PATHOGENS

Laboratory analyses in Paper I-III were performed by routine assays at the Clinical Virology laboratory. Respiratory sampling of patients was made at the discretion of the treating physician, mainly by nasopharyngeal swabs (FLOQSwabs™ in Paper I and Eswabs™ in Paper II, COPAN Industries Inc) and occasionally by bronchoalveolar lavage. No additional sampling of patients was made for the studies. Clinical samples were stored in the laboratory and frozen at -20°C after routine analysis.

The multiplex inhouse qPCR method used for diagnostics has previously been described in detail [91]. It has been increasingly used since the introduction in 2006 and currently includes 17 respiratory pathogens. The following pathogens are included: influenza A and B, respiratory syncytial virus, human rhinovirus, coronavirus (NL63, OC43, 229E and HKU1), metapneumovirus, adenovirus, bocavirus, parainfluenza virus type 1-4 and five bacterial agents: *S pneumoniae, H influenzae, C pneumoniae, M pneumoniae* and *B pertussis.* The test is run once a day Monday-Saturday with a turnaround time of 12-24 h. In short, nucleic acid from 100 µL specimen are extracted into an elution volume of 100 µL and amplified in 25 µL reaction volumes. After reverse transcription, 45 cycles of two-step PCR is performed. Each sample is amplified in 8 parallel reactions containing primers and probes specific for 2-4 target agents. A cycle threshold (Ct) <40 is considered as a positive result.

Clinical testing of hospitalized patients with symptoms of respiratory infection is common with a current number of ~13 000 analyses/year. PCR data were included in paper Paper I-III. Viral load was expressed as Ct values, where a high Ct value represent a low viral load.

6.3 CONTROL MEASURES

Infection control recommendations for suspected influenza cases (Paper II-IV) include care in a single occupancy room and personal protective equipment for standard and droplet precaution (surgical mask combined with glasses or a full-face visor) for HCWs.

Chemoprophylaxis for influenza (75 mg oseltamivir once daily for ten days) was recommended for exposed patients (Paper II-III) regardless of vaccination status. According to national guidelines, antiviral treatment (75 mg oseltamivir twice daily for five days) should be considered for patients with severe influenza or a high risk of complications (specified as all patients needing in-hospital care).

6.4 DEFINITIONS IN PAPER II-IV

An influenza case was defined as laboratory confirmation of influenza virus in a respiratory sample by multiplex real-time PCR in addition to symptoms of ILI or ARI. Influenza-like-illness (ILI) was defined as stated by CDC as fever >37.8 ^{o}C and cough or sore throat. Acute respiratory infection (ARI) was defined as sudden onset of cough, sore throat or shortness of breath regardless of fever with no other plausible cause. Exposure was defined as contact by sharing room at a hospital ward with an influenza case. Healthcare-associated influenza infection (HCAI) was defined as onset of ILI/ARI >48 hours after hospital admission or <48 hours after a previous discharge [100]. Morbidity was expressed as Charlson co-morbidity score (CCI) [123].

6.5 ETHICAL CONSIDERATIONS

The Regional Ethical review board in Gothenburg approved the studies in Paper II-III. No ethical approval was needed in Paper I, as analyzed samples had been collected prior to our study and no clinical or personal data was included. This also apply for Paper IV.

6.6 METHODS PAPER I

6.6.1 Subjects

The study cohort for Paper I includes clinical respiratory samples positive for rhinovirus by real-time PCR. Samples from 170 patients were selected which represent approximately 10% of the total amount of samples positive for rhinovirus from November 2006 through September 2010. No patient data were included.

6.6.2 Design

Stored respiratory samples were selected to represent both autumn and spring across four consecutive seasons. The obtained sequences from local samples were compared with reference sequences from other geographical areas representing known HRV types. These references included 74 HRV-A, 24 HRV-B and 50 HRV-C sequences, classified as suggested by the International Committee on Taxonomy of Viruses (ICTV) Picornaviridae Study Group (with provisional classification for 14 HRV-C sequences). In order to retrieve the 5–10 published sequences of the same type with the closest similarity, a BLAST search was performed for each of our sequences.

6.6.3 Typing, sequencing and phylogeny

All 170 samples were selected for sequencing of the VP4/VP2 regions followed by phylogeny if amplicons were of sufficient length and quality. After total nucleic acid extraction in a MagNA Pure LC instrument (Roche, Branchburg, NJ, USA), amplification was performed using the primers Rhino_547F and Rhino_1125R, in a first PCR and Rhino_547F and Rhino_1087R, in a second PCR. Cycle sequencing was carried out in both directions using ABI BigDye Terminators (Life Technologies, Carlsbad, CA, USA) and Rhino_547F and Rhino_1087R as primers, and the sequences were read in an ABI 3130XL instrument and assembled using the Lasergene software (DNASTAR, Inc., Madison, WI, USA).

A segment of 395 nucleotides were aligned along with reference sequences and phylogenetic trees were constructed by maximum-likelihood analysis using MEGA® Version 5.0 software. Type assignment was based on a >90% nucleotide similarity to a reference sequence or clustering with a with a reference sequence in the phylogenetic analysis with a bootstrap value >70%. Genetic distances between and within types were compared by Student's t-test.

6.7 METHODS PAPER II

6.7.1 Subjects

The outbreak studied in Paper II consisted of 20 patients with influenza B virus infection at Kungälv hospital, Sweden, during a period of six weeks in May-June 2016. The report includes all patients with a respiratory sample positive for InfB during an extended time period which precedes the admission of the index case of the outbreak by one week and terminates one week after confirmation of the final case. This constitutes 67% of all samples positive for InfB at the laboratory during the study period. All patients admitted to the main affected ward during the outbreak were also evaluated in order to find cases of influenza not detected by the laboratory.

6.7.2 Design

Retrospective review of medical records was conducted, and the following variables were registered: dates for admittance and discharge, type of ward, wardroom, respiratory sampling date, age, sex, co-morbidities, antibiotic treatment and whether the influenza infection could be classified as HCAI. A putative map for transmission was created by using both genetic and patient data in relation to time and location within the hospital.

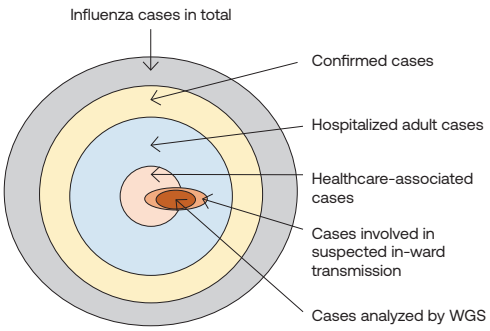
6.7.3 Typing, sequencing and phylogeny

Stored respiratory samples were selected for lineage typing along with phylogenetic analysis of the full-length hemagglutinin (HA) gene. InfB detection and lineage typing (B/Yamagata or B/Victoria) was performed by real-time PCR using the TaqMan Fast Virus 1-Step Master Mix (Applied Biosystems/Thermo Fisher Scientific, Carlsbad, CA, USA) and the 7900HT Fast Real-Time PCR System (Thermo Fisher Scientific) by the Department of Microbiology, Unit for Laboratory Surveillance of Viral Pathogens and Vaccine Preventable Diseases, Public Health Agency of Sweden, Stockholm.

The RT-PCR products were sequenced using the Ion Torrent S5 XL (Thermo Fisher Scientific) platform. The sequencing reads from Ion Torrent were mapped against B/Phuket/3073/2013 (EPI_ISL166957, downloaded from the GISAID EpiFlu Database, www.gisaid.org) in CLC Genomics Workbench (Qiagen). The phylogenetic tree was constructed from aligned full-length haemagglutinin sequences along with all Swedish B/Yamagata strains collected and sequenced during season 2015/2016, the vaccine strain for northern hemisphere season 2015/2016 and reference strains.

A phylogenetic tree was constructed using the maximum-likelihood method in Mega® Version 5.1. Bootstrap values were obtained from 1000 replicates and displayed on nodes if >70%. In addition, a detailed analysis of nucleotide differences within the entire InfB genome of the outbreak strains were performed. To reveal single nucleotide variants, all nucleotide sequences (coding region) from the 18 cases were aligned with each other in CLC Genomics Workbench.

Figure 8: Illustration of the hospital influenza population



6.8 METHODS PAPER III

6.8.1 Subjects

The study in Paper III included all hospitalized patients ≥18 years old with a positive respiratory sample for InfA during the study period from July 1st, 2016 to June 30th, 2017 at Sahlgrenska University Hospital. Altogether 435 patients were included, which constituted 45% of the total amount of influenza positive samples analyzed at the Clinical Virology laboratory during the time period. Only cases where respiratory sampling was performed at patients admitted at a hospital ward or at the ED followed by admission of the patient were included. A schematic overview of the hospital influenza population is displayed in Figure 8.

6.8.2 Design

Retrospective review of medical records was conducted and following variables were registered: age, sex, co-morbidity, time of sampling, onset of symptoms, antiviral therapy, length of stay, type of ward, 30-day mortality, and whether the influenza infection was classified as a HCAI.

Univariate survival analysis comparing HCAI and non-HCAI cases was performed using the log-rank (Mantel-Cox) test. Multivariable Cox proportional hazard regression model was used to further explore the covariates and P-values < 0.05 were considered statistically significant. The model used the backward stepwise (Wald) method and hazard ratios above 1 indicated a positively associated covariate. Statistical analyses were performed using the SPSS software package, version 25 (IBM, Armonk, New York, US).

In-ward transmission was suspected when two or more patients tested positive for InfA in samples collected at the same ward within 7 days. All cases involved in possible in-ward transmission were selected for lineage typing and whole-genome sequence analysis.

6.8.3 Typing, sequencing and phylogeny

Lineage typing and sequence analysis were performed by laboratory staff blinded for epidemiological data. RT-PCR products was used in library preparation performed by AB Library Builder system (Applied Biosystems). Each genome library of about 300-bp fragments was quantified with the Ion Library TaqMan Quantitation Kit (Thermo Fisher Scientific) and template preparation was performed by the Ion Chef system (Thermo Fisher Scientific).

Sequencing was performed using the Ion Torrent next generation sequencing platform with the reference sequence for H3N2 accessed from GenBank. Bioinformatic analysis was performed with the web-based platform INSaFlu and consensus sequences of each InfA genome were obtained [113]. For comparison, samples obtained at primary healthcare centres in the same region, during the same season, were also included. A phylogenetic tree was constructed using the maximum likelihood method in Mega® Version 7. Bootstrap values were obtained from 500 replicates and displayed on nodes if >70%.

6.9 METHODS PAPER IV

For Paper IV, data regarding patient flow and clinical management from Sahlgrenska University Hospital, Gothenburg, Sweden was used to constitute the base of a system dynamics model of in-hospital influenza transmission. A simple flowchart illustrating the patients' way from the ED through the hospital until discharge is shown in Figure 9.

6.9.1 Design

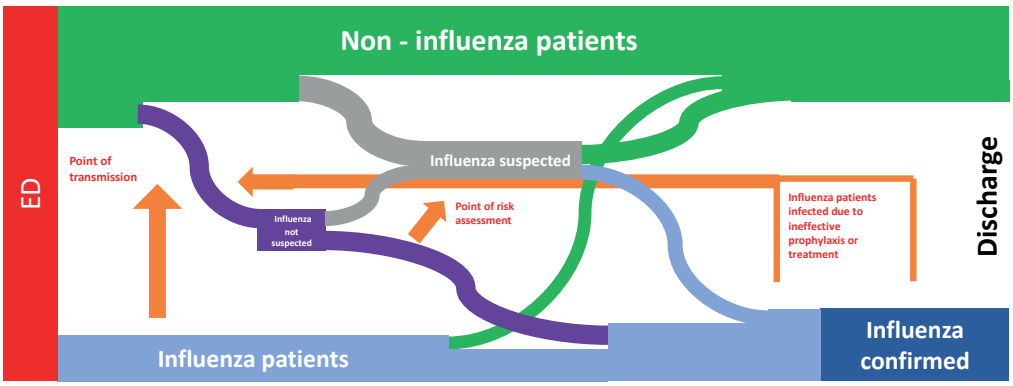
The SD model was designed exclusively for this study and integrates local hospital data with virologic properties and national surveillance data. A detailed description of the construction of the model can be found in Paper IV. It enables quantifications of scenarios by mathematical expressions and interactions where both actual data and assumptions can be combined. We used the data to construct a model of a typical hospital, followed by producing seasonal estimates of the number of HCAI influenza cases by simulating future plausible scenarios.

The modelling process consisted of the following consecutive steps:

(1) Identifying key variables with a potential influence on in-hospital transmission of influenza.
(2) Construction and technical validation of the model.
(3) Selecting the model scenarios of interest.
(4) Producing the SD simulations.

Multiple stepwise simulations were then performed in order to identify potential control strategies with high benefit in order to reduce in-hospital influenza transmission. Construction of the model was made in collaboration with Paul Holmström and Stefan Hallberg with long time experience in systems thinking and simulation development. The Stella Architect simulation software (Stella Architect®, version 1.7.1, isee systems Inc, Lebanon, NH, USA) was used.

Figure 9: Flow chart of the patient populations



RESULTS AND DISCUSSION

7 RESULTS AND DISCUSSION

7.1 RESULTS PAPER I

In this retrospective study, 114/170 (67%) of selected clinical samples positive for rhinovirus by real-time PCR produced sequences of sufficient length and quality for phylogenetic comparison. In 54/114 cases (47%), the samples were obtained from children <18 years old and 56/114 (49%) were obtained from females.

7.1.1 HRV types

By sequence analysis of the VP2/VP4 region we found in total 64 HRV-A, 11 HRV-B and 37 HRV-C types. There were 33 different subtypes of HRV-A, 9 HRV-B and 37 of HRV-C and some types were found across several seasons.

7.1.2 Phylogenetic analysis

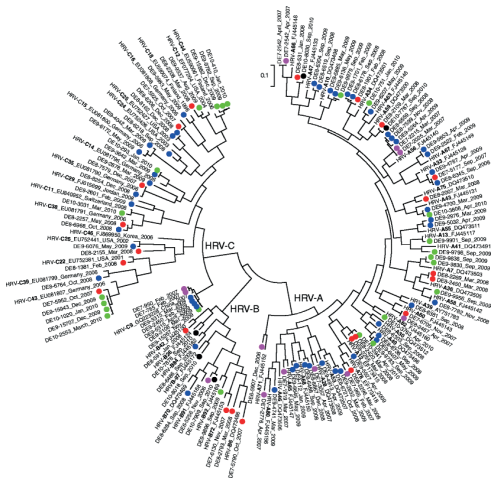
The mean nucleotide difference was 39.3% between HRV-A and HRV-B, 38.5% between HRV-A and HRV-C, and 40.2% between HRV-B and HRV-C. The variability within the HRV-C strains was greater (24.4%) than within HRV-A (20.3%, p<0.0001) and HRV-B (21.1%, p= 0.0002) strains.

All HRV sequences included in our investigation along with the reference sequences are presented in a phylogenetic tree, Figure 10. The tree reveals that some closely related subtypes appeared during two or three seasons, suggesting circulation in the population over long time periods. To further explore this, we constructed separate phylogenetic trees for each of these types in comparison with ~10 related sequences retrieved from Genbank. These trees demonstrate examples of greater as well as less similarity between our strains of the same subtype when compared with related sequences from other parts of the world. However, the majority of the closely related sequences had been collected the same or previous/following year.

7.1.3 Putative new types

One HRV-B and six HRV-C sequences showed less than 85% nucleotide similarity with the reference sequence. This suggest that they might represent new subtypes. For each of these cases there was at least one published sequence with >90% similarity, but type assignment could not be defined for as analyze of VP1 is required [124].

Figure 10: Phylogenetic tree by maximum-likelihood analysis of 112 HRV sequences from the present study and database reference sequences (in bold). The coloured dots indicate the sampling season: pink, 2006/2007; red, 2007/2008; blue, 2008/2009; green, 2009/2010; black 2010



7.2 DISCUSSION PAPER I

In Paper I, we observed a wide spectrum of HRV subtypes each season. Different subtypes also appeared during successive seasons. The genetic diversity between and within the subtypes may contribute to the seasonal pattern of HRV and the ability to prevail across seasons. Despite the limited sample size of our study, it supports to some extent the hypothesis that HRV may cause restricted outbreaks in a time-limited fashion, similarly to other respiratory viruses.

Although each HRV subtype may appear during a limited time period, the identification of some types from successive seasons points at the possibility of more extended periods of circulation. The reason for this is probably multifactorial, possibly influenced by prolonged viral shedding, mild clinical presentation (which allows HRV infected subjects to be more likely to expose others) and a robust unenveloped virion structure [125].

Our study does not represent an extensive survey, but a judgement sample of HRV in different types of patients during a long time period and defined geographical area. A larger number of HRVs would have to be sequenced to illustrate the pattern of circulating subtypes more adequately. The observed proportions of HRV type A-C is however in line with other reports following this publication [126-128] as well as co-circulating of strains and potential severity of clinical presentations associated with HRV infections [129].

For classification, phylogeny based on sequencing of the VP1 region has been more reliable than the VP2/4 region being used in our study. For HRV-A and HRV-B, sequencing of VP2/4 has been shown to correlate well with VP1 and serological classification [130, 131]. No serological typing technique is available for HRV-C, and classification is based only on sequence comparison with a divergence of more than 13 % in VP1[124]. New HRV-C subtypes could therefore not be identified in our investigation.

In summary, HRV is a diverse pathogen with a wide spectrum of subtypes. Further studies are needed which include sequencing of many strains, longer duration and including asymptomatic patients to clarify the detailed seasonal and global transmission pattern. This may in the future contribute to explain to the successfulness of HRV.

7.3 RESULTS PAPER II

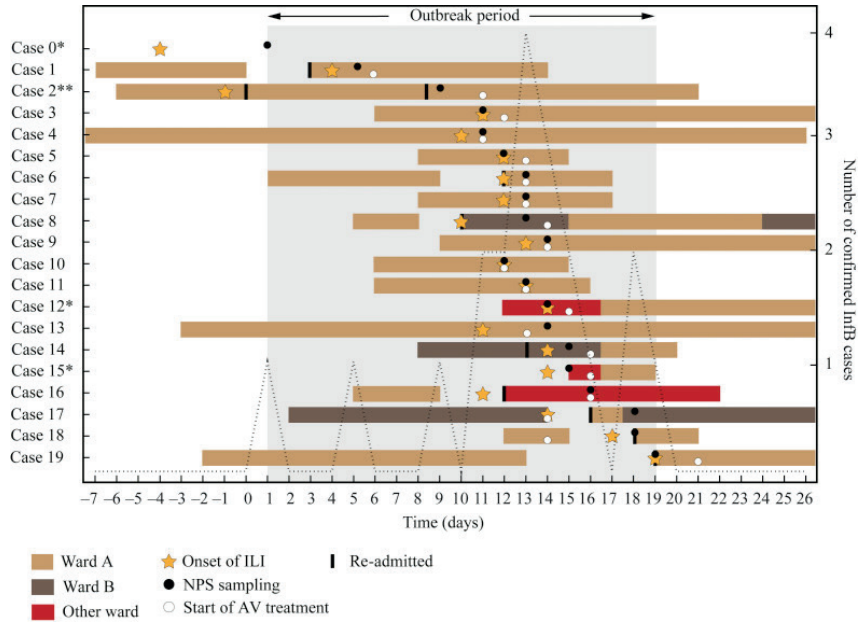
In this retrospective study of a hospital outbreak, 17/20 of patients with influenza B during a period of four weeks could be linked to each other by either shared room or shared ward. In 15/17 of these cases, WGS was successful (or partially successful) and strongly supported the epidemiological link.

7.3.1 Outbreak

The index case (Case 1) was a 66 year old male where the ED nurse noted that the patient's wife had ILI. He developed fever and respiratory symptoms four days after admission, underwent sampling day five, and was moved to a single room and received oseltamivir treatment on day six.

In order to find possible links to the outbreak, all positive Inf B samples over an extended time period were evaluated. This period precedes the admission of the index case by one week and terminates one week after confirmations of the final case. We found one patient (Case 0) sampled at the ED two days before admission of the index case. No other epidemiological links from Case 0 to the other patients involved were found. An overview of the outbreak is shown in Figure 11.

Figure 11: Overview of all confirmed Inf B cases from the hospital during an extended time period. Location, onset of ILI/ARI in relation to NPS and initiation of antiviral treatment are shown. The defined outbreak period range between NPS sampling day of case 0 and 20.



* Case 0, 12 and 15 could not be linked to the "true" outbreak, starting with the index patient at ward A.

** Case 2 developed diffuse respiratory symptoms meeting the criteria for ARI ten days before NPS sampling, and in addition also had a high CT value. Clinical picture and time of InfB infection are in this case unclear.

7.3.2 Outcome

During the outbreak period, 19/75 patients admitted to the most affected ward (Ward A) were diagnosed with Inf B resulting in an attack rate of 25%. The median age of patients was 77 years old with a mean length of hospital stay (LOS) of 11.3 days. Median CCI score was 4. The cycle threshold (Ct) value indicated a high viral load in most cases. In ward A, 15 HCWs reported sick-leave due to fever and respiratory symptoms between day 8 and 19.

7.3.3 Molecular characterization of viral isolates

Phylogenetic tree of all HA sequences is shown in Figure 12. A high Ct value prevented sequencing in one case and in one case no sequence was obtained.

All the 18 sequenced strains belonged to Influenza B/Yamagata, genetic clade 3. Fifteen of the 18 cases had identical HA sequences, although one case contained a mix of two nucleotides in one position. The remaining three cases had identical HA sequences but differed in three nucleotide positions from the other 15 cases. All 18 cases were identical at amino acid level and differed from all other Swedish Influenza B/Yamagata strains collected and sequenced during season 2015/16.

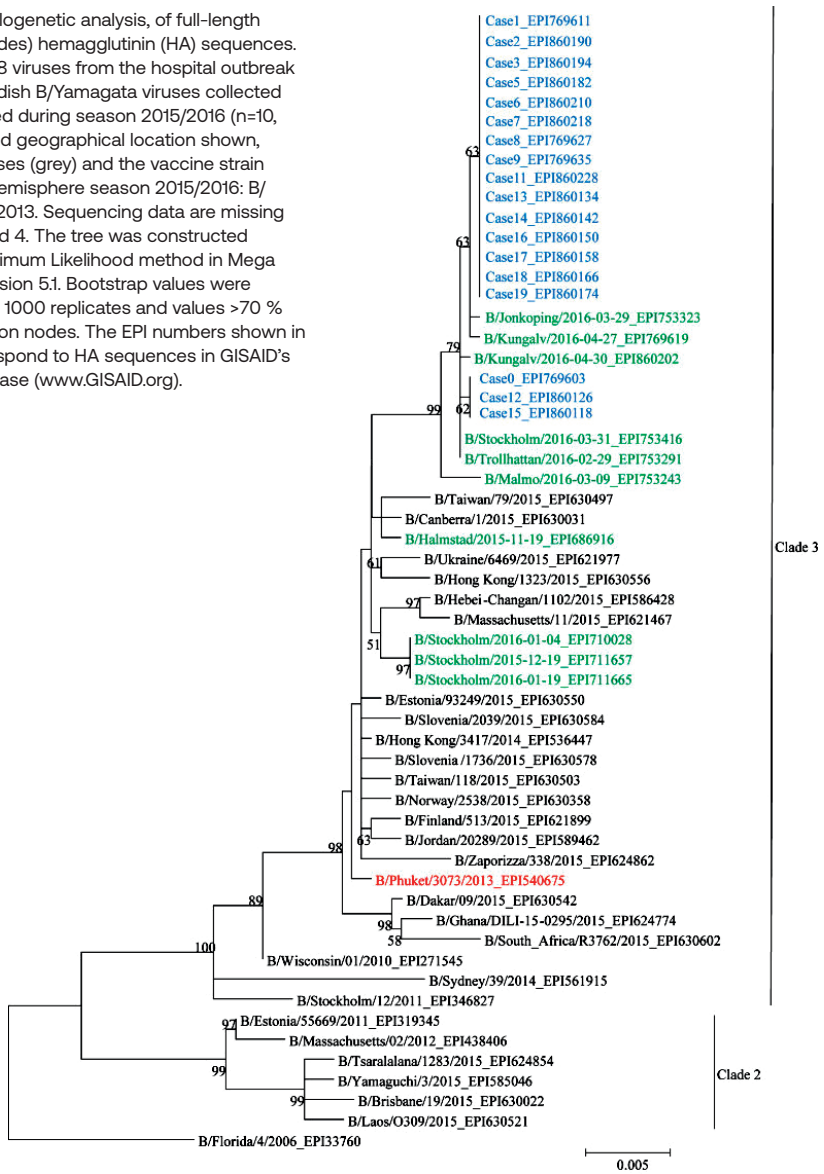
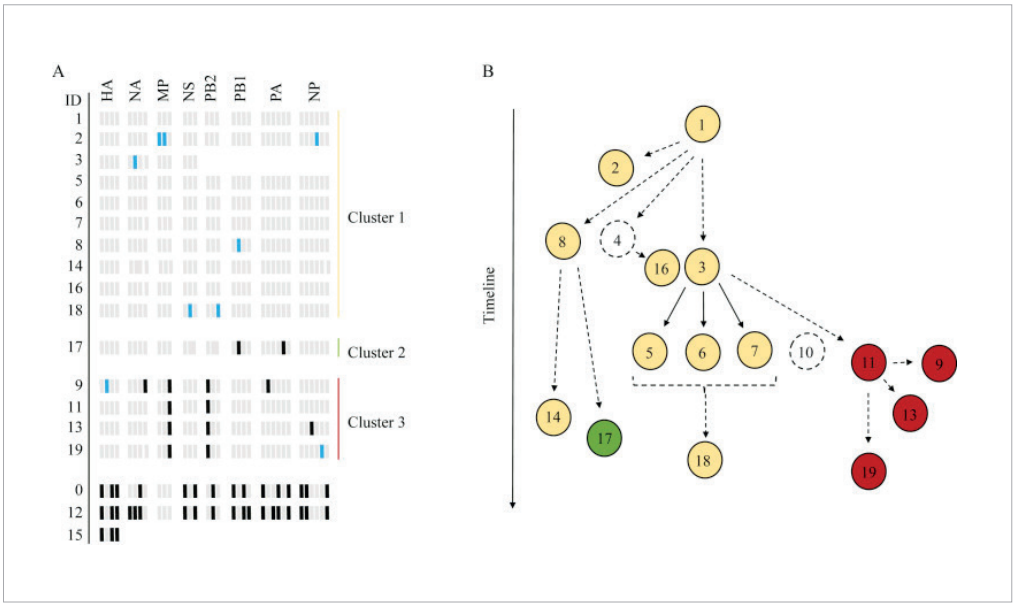


Figure 12. Phylogenetic analysis, of full-length (1755 nucleotides) hemagglutinin (HA) sequences. Included are 18 viruses from the hospital outbreak (blue), all Swedish B/Yamagata viruses collected and sequenced during season 2015/2016 (n=10, black) date and geographical location shown, reference viruses (grey) and the vaccine strain for northern hemisphere season 2015/2016: B/Phuket/3073/2013. Sequencing data are missing for case 10 and 4. The tree was constructed using the Maximum Likelihood method in Mega ® software version 5.1. Bootstrap values were obtained from 1000 replicates and values >70 % are displayed on nodes. The EPI numbers shown in the tree correspond to HA sequences in GISAID's EpiFlu™ Database (www.GISAID.org).

Analysis of nucleotide differences within the entire genome could arrange the strains in three clusters. A putative transmission map was created using nucleotide and patient data in relation to time and location within the hospital. The map (shown in Figure 13) highlights the complexity of outbreak progression.

Figure 13 A: Single nucleotide variants identified in the eight segments of the sequenced InfB genomes. B. Putative map for InfB transmission based on SNV analysis of the whole InfB genome and patient overlap within a ward. Nodes represent cases and arrows indicate transmission events, directly or directly from one patient to the other.



7.4 DISCUSSION PAPER II

In Paper II, the hypothesis of in-hospital transmission was supported by molecular data which identified one virus strain as the cause of multiple secondary cases. Recent advances in molecular biology has yielded new insights in transmission dynamics, which may be used to either corroborate or convene classic epidemiological links [132]. WGS has made detailed investigations of single nucleotide variants (SNV's) possible, which in our study was found to be in line with the mutation rate for InfB [44, 133]. This indicated that changes occurred within the influenza genome during the outbreak and made it possible to create a putative transmission map.

The ability to detect the starting point of an outbreak may be challenging in a dynamic environment with high density of patients. An acute-care facility has a constant in- and outflow of patients, and the index case is not necessarily the true primary case [134]. All big outbreaks start off as small outbreaks – and adequate timing of preventive measures is crucial. In our study, a local outbreak was not suspected until day 13, when already seven InfB cases were confirmed. Delayed initiation of control measures in relation to onset of symptoms in the beginning of the outbreak may have enabled the virus to spread efficiently within the hospital. Swift responses are particularly important to prevent further transmission when it comes to infectious agents with short incubation periods, such as influenza [57].

Based on our findings, we suggest that InfB may spread efficiently to patients not characterized as

being exposed according to current infection control guidelines for the hospital. Defining true exposure is difficult, especially when unrecognized sources of infections are suspected to be involved. Moreover, limiting the definition only to patients sharing room may not be enough, as intra-hospital transfer of patients is common. The relative importance of different modes of transmission for influenza is not clear. Multiple studies [9, 135, 136] have provided evidence for the importance of aerosol transmission, why exposure should be defined with care.

The attack rate in our study was 25% for the most affected ward (ward A) and 12% of patients admitted during the outbreak was given antiviral prophylaxis with oseltamivir. Attack rates reported in influenza outbreaks ranges between 1%-65% with an adjusted mean of 28% [137], but are highly dependable on case definitions and settings.

One limitation is that additional data regarding number of possibly exposed cases or information regarding HCWs from wards at the hospital other than ward A was not investigated. Only one probable case with ILI/ARI symptoms without verified infection was identified at ward B which indicates a low threshold for sampling of patients. In contrary for HCWs, no sampling was performed for the 15 unvaccinated members of the staff reporting sick-leave during the outbreak. Their role therefore remains unclear, both in terms of direct transmission to/from patients and indirect in aspect of adherence to control measures.

Further limitations are a lack of data regarding vaccination status for involved patients. Even though the outbreak strain was included in the seasonal vaccine, the protective effect of vaccination was probably very limited since the outbreak occurred in May/June. Antibody titers peak 2-4 weeks after vaccination [138] and is followed by a significant decline after 180 days [139]. Several unknown factors such as detailed contact data and unrecognized cases may further have affected the course of the outbreak and the putative transmission map.

7.5 RESULTS PAPER III

In this retrospective study, all adult hospitalized patients with confirmed influenza A infection during season 2016-17 were included. Extensive in-ward clustering was revealed, and healthcare-associated influenza was identified as possibly having a more severe outcome. A flow chart of the study population is shown in Figure 14.

7.5.1 Patient characteristics and outcome

We identified 435 InfA cases of which 114/435 (26%) were classified as HCAI. The overall 30-day mortality rate was 6.0% (n=26/435) and 7.2% (n=24/333) among patients ≥65 years old. The 30-day mortality rate was higher among patients in the HCAI-group compared with the non-HCAI group, see Figure 15.

Among the patients who died within 30 days, respiratory causes were predominant, accounting for 5/15 (33%) deaths in the non-HCAI and 7/11 (63%) in the HCAI group. Cardiovascular events were also common. Antiviral treatment was given in 7 out of 15 cases (47%) for patients in the non-HCAI group and in 6 out of 11 (55%) in the HCAI group. In multivariable Cox regression analysis, only age remained an independent predictor of death within 30 days after respiratory sampling. Although having a healthcare associated influenza did not reach statistical significance, it was noted as a potential risk factor for death (p=0.082). No cases were lost to follow-up.

Figure 14: Flow chart of the patient population. Grey boxes represent cases selected for in-ward transmission analysis.

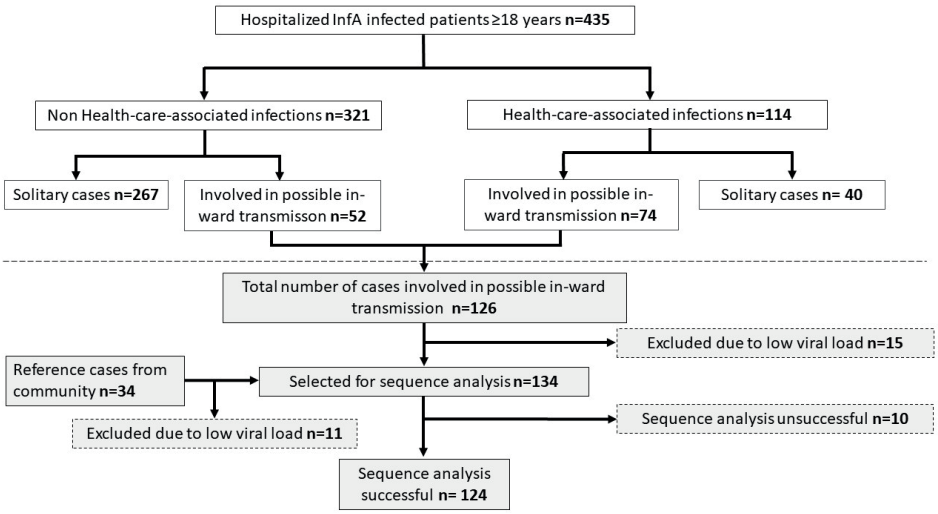
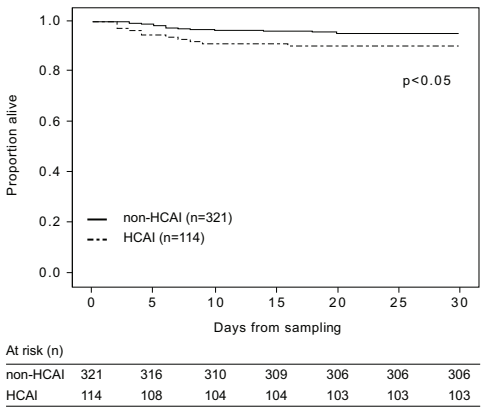


Figure 15: Kaplan-Meier plots showing survival curves for non-healthcare associated (non-HCAI) and healthcare-associated (HCAI) InfA cases.



At risk (n)

non-HCAI	321	316	310	309	306	306	306
HCAI	114	108	104	104	103	103	103

Of cases classified as HCAI, 74/114 (65%) were possibly involved in in-ward transmission. In another 40 cases, defined as HCAI, no additional InfA case could be identified at the same ward within 7 days. In the non-HCAI group, 52/321 cases (16%) were involved in possible in-ward transmission as possible primary cases.

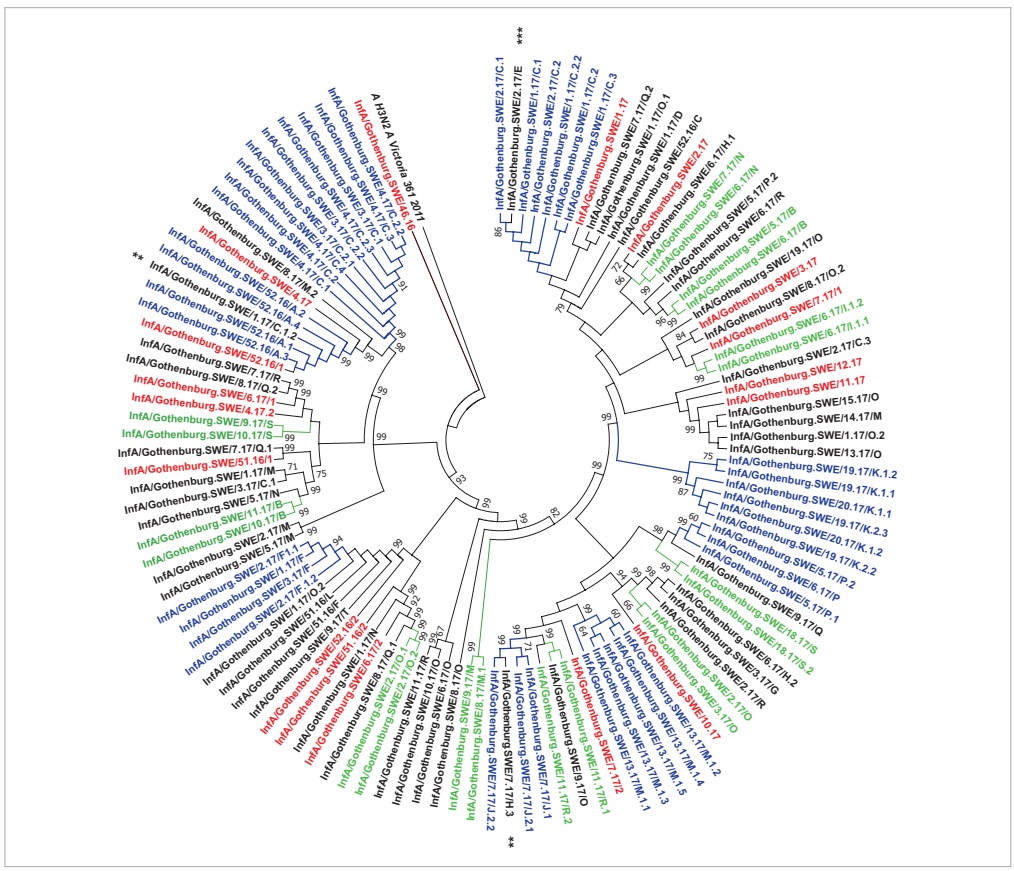
If more conservative HCAI-criteria were used (onset of symptoms <72 hours after admission or <24 hours after discharge when readmitted), the proportion of HCAI still remained high at 22%. Median time from admission to symptom onset was 8 days, and in 55 cases (48%) onset occurred after >7 days of hospital care.

7.5.2 Molecular characterization of viral isolates

All InfA samples were of subtype H3N2 and WGS was successful in 124/134 (93%) of the hospital cases selected for in-ward transmission analysis. Altogether 60/124 (48%) of the sequenced samples belonged to an in-ward cluster or pair. Figure 16 shows the phylogenetic tree based on WGS data, which identified eight separate clusters (involving ≥3 strains) and another ten pairs of strains from cases related in time (interval ≤7 days) and location (shared ward).

WGS also revealed a close relationship between an in-ward cluster and a single strain from another ward in three cases. Detailed analysis of possible transmission events revealed adjacent localization of wards in two of these cases and recent transfer from an affected ward in one case. Strains obtained in primary healthcare were dispersed throughout the tree.

Figure 16: Phylogenetic analysis of selected InfA strains based on WGS compared with the H3N2 reference strain in italic. Names correspond to InfA/city/country/week/year/ followed by letters A-S representing ward and serial number. Strains showing in-ward transmission clusters are indicated (blue), in-ward pairs (green) and background sequences (red). Asterisks show strains closely related to a cluster but from separate wards. The tree was generated by using the maximum likelihood method in Mega7 version 5.1. Bootstrap values were obtained from 500 replicates and values >70 % are displayed on nodes.



7.6 DISCUSSION PAPER III

In Paper III, we present the clinical characteristics of adult patients hospitalized with influenza and we show how WGS may be used to investigate in-ward transmission.

Reliable identification of cases involved in transmission is impossible without laboratory confirmation. As PCR-methods are becoming increasingly available, earlier detection by the treating physician and higher diagnostic accuracy is achieved. Likewise, outbreak investigations have previously relied upon a traditional workflow based on case definitions, case confirmations, determination of the background rate and identification of epidemiological links. In the new era of sequencing, surveillance of communicable diseases is reshaping and allows for more precise investigations. Viral sequencing in cases involved in hospital outbreaks has previously often shown non-related strains [140, 141]. In our study, the extensive phylogenetic in-ward clustering based on the selection of epidemiologically related cases strongly support the suspected transmission. A closer inspection of the sequences also revealed low genetic diversity within, and distinct separation between, the individual clusters.

We classified 26% of the InfA cases as HCAI, which is higher compared with several previous reports [142-144]. It is important to bear in mind that this definition is not equal to a proven case of hospital-acquired influenza. We used the most common definition of a health-care associated influenza [100] in order to compare the HCAI and non-HCAI patient groups. For the purpose of reliable identification of hospital transmission, we instead included local and temporal proximity in addition to phylogenetic analysis. By this mean, possible index cases in the non-HCAI group (for example cases not recognized as influenza upon admission) were able to be included in the in-ward transmission analysis.

By dividing the InfA cases into two groups of HCAI and non-HCAI, comparison of patient characteristics could be made. We found that InfA patients categorized as HCAI had a longer total length of hospital stay and were more likely to die within 30 days of sampling compared with the non-HCAI group. However, only age remained as an independent risk factor for death in the multivariable regression analysis. The CCI index used for estimating morbidity might be less suitable for influenza. We suspect there is a higher vulnerability due to other medical conditions in the HCAI group which is not captured by the CCI scoring system. This is illustrated partly by a median of eight days of hospital stay from admission to symptom onset in this group. Recent findings have also shown increased risk of severe laboratory-confirmed influenza for adults with specific chronic medical conditions [75].

Several unknown factors may be of importance but not considered in our study. No information regarding influenza vaccination in patients or vaccination or symptoms for HCW were accessible. Detailed contact data beyond shared ward were lacking. The total number of patients exposed to an influenza case were lacking. No calculation of attack rate or estimation of protective effect of antiviral prophylaxis could be made. No information of adherence to infection control measures were available. Documentation regarding exact time of symptom onset were sometimes lacking, why we chose time of sampling to compare the 30-day survival between the HCAI and non HCAI-group. This also makes identification of primary cases and detailed analysis of outbreak progression impossible.

In summary, although data were collected retrospectively and are incomplete, this study illustrate how influenza effectively may spread within hospital wards. Future evaluation by hospital managements of patient flows and effective measures for influenza control is needed to protect vulnerable patients.

7.7 RESULTS PAPER IV

7.7.1 Model construction

Our SD model was based on the involved patients flows within a hospital, where a non-influenza infected patient population is infected by an influenza infected population. The resulting number of HCAI-cases further depend on infectivity and exposure. The model enables quantifications of scenarios by mathematical expressions and interactions where both actual data and assumptions can be combined.

7.7.2 Simulations

In order to identify the most effective control measures for a hospital to reduce the number of HCAI cases of influenza per season we first concentrated on modifiable patient-related factors. Model scenarios in the first simulation round was stepwise altered as followed:

(1) Mean number of patients exposed by shared room/ influenza case.
(2) Share of non-HCAI cases receiving antiviral treatment within 48 h of symptom onset.
(3) Share of HCAI influenza cases receiving antiviral treatment within 48 h of symptom onset.
(4) Share of exposed patients receiving antiviral prophylaxis.

One variable at a time was given a set value and outcome is presented as the estimated total number of HCAI cases per season.

In the second simulation round, the two patient-related variables identified as having the most impact were retained and scenarios beyond hospital control (i.e. non-modifiable) were added followed by stepwise alteration of:

(1) Vaccine coverage.
(2) Vaccine effectiveness.
(3) Total number of patients seeking care at the ED with symptoms of possible influenza per season.

Variables altered in simulation round 1-2 are summarized in Table 1.

7.7.3 Outcome

Antiviral prophylaxis given to patients who were exposed by sharing room with an influenza case was identified as the single most effective measure, followed by a reduction of the mean number of exposed patients. Antiviral treatment of symptomatic non-HCAI, as well as of HCAI cases, had limited effect on in-hospital transmission.

The impact of antiviral prophylaxis initiated after exposure found in our model was well demonstrated by an estimated number of HCAI of less than 100 in spite of a worst case model scenario including variables set to 0% vaccine coverage, 0% vaccine effectiveness, a mean number of 3 exposed cases/ influenza case or a total inflow of 2000 patients with influenza symptoms to the ED.

7.7.4 Additional results

We further estimated the risk of contracting influenza during hospital stay and compared this with those applied for different model scenarios. Based on the hospital data from 2016-17, following calculations were made.

The influenza season was assumed to last for 12 weeks. The total number of patients admitted during this season was estimated to be 3588 (on average 4 600/month ED appointments with an

Table 1. Basic model variables and altered variables in simulation round 1 + 2

Basic model variables	
Influenza cases (n)	435
Mean number exposed in shared rooms (n)	2.2
Vaccine coverage (%)	49
Vaccine effectiveness (%)	40
Share of exposed treated with prophylaxis <48 h (%)	56
Prophylactic effectivity (%)	80
Diagnostic accuracy at ER (%)	56
Share of non-HCAI influenza treated on admission (%)	53
Share of HCAI influenza treated <48h (%)	62
Variables modified in simulation round 1	
Mean number exposed in shared rooms (n)	1- 2- 3
Share of non-HCAI treated on admission (%)	0-25-50-75-100
Share of HCAI treated <48 h (%)	0-25-50-75-100
Share of exposed receiving prophylaxis (%)	0-25-50-75-100
Variables modified in simulation round 2	
Mean number exposed in shared rooms (n)	1- 2- 3
Share of exposed receiving prophylaxis (%)	0-25-50-75-100
Mean vaccine coverage (%)	0-25-50-75-100
Mean vaccine effectiveness (%)	0-25-50-75-100
Total influenza inflow to ER (n)	500-1000-1500-2000

admittance rate of 26%). The number of non-HCAI cases were found to be 321, which leaves a total of 3588-321 = 3267 patients at risk of acquiring influenza during hospital stay. The number of HCAI cases were found to be 114, which leaves an estimated risk for patients not infected on admittance to develop influenza during hospital stay of 3.5%.

If all other variables were unchanged, by increasing the share of prophylaxis from 0-100%, the risk for contracting influenza decreased as followed: Mean number of exposed cases one: 2.8-0.5% two: 7.2-1.1% and three: 13.2-1.7%. Future scenarios selected for risk calculations were: Mean number (1-3) of exposed patients in shared rooms in relation to share of exposed patients receiving antiviral prophylaxis (0-100%).

In Table 2, the absolute and relative risk reductions are displayed in addition to relative risk and number of patients needed to treat to prevent one HCAI case.

Table 2. Risk reduction for HCAI influenza shown for mean number of exposed cases (1-3) in relation to effect of increasing the share of exposed receiving prophylaxis (0-100%)

Mean exposed (n)	HCAI (n) Prophylaxis 0%	HCAI (n) Prophylaxis 100%	ARR	RRR	RR	NNT
1	92	17	0.02	0.81	0.19	45
2	235	33	0.06	0.85	0.15	18
3	432	54	0.10	0.86	0.14	10

ARR: Absolute risk reduction, RRR: relative risk reduction, RR: relative risk and NNT: Number needed to treat

7.8 DISCUSSION PAPER IV

In Paper IV, we present a system dynamic model for illustrating healthcare-associated influenza at a typical hospital. We further use the model to make predictions of future scenarios and estimate the effect of preventive interventions.

Modelling in general, and perhaps SD modelling in particular, may be perceived as abstract to users not familiar with the technique. It is important to bear in mind that all simulated data are approximations, based on assumptions with different levels of uncertainty. Standard statistical methods, in which evidence is based on significance, do not apply for system dynamics. Instead, the advantage is a possibility to supply approximations for interpreting reality.

Although all models use simplifying assumptions, a model needs to depict the real-world as close as possible in order to be valuable for users. In our model, this is enabled by adding local hospital data, national surveillance data, and by the possibility to include any new scenario and modify any variable when new data becomes available. This will allow the model to continually improve.

The finding of antiviral prophylaxis as an effective measure to reduce the number of HCAI cases in our model is in line with previous reports [145, 146]. However, the assumed association between infectivity and nasopharyngeal viral load might lead to an overestimation of transmission occurring around the time of symptom onset [147].

Hospitalization in double-occupancy rooms vs single-occupancy rooms has been associated with a higher risk of hospital-acquired influenza in a prospective cohort study [148]. The low impact of antiviral treatment of already symptomatic patients to prevent transmission which was detected by our model is also supported by other reports [145]. It is also important to bear in mind that the aim of our model is to specifically illustrate nosocomial transmission of influenza on a hospital level. Risks and benefits of antiviral treatment or other control measures may be present for the individual patient, even of little relative importance for decreasing onward transmission.

Another concern is the "testing one variable at a time" - strategy. A more likely envision of future scenarios is that several control strategies for influenza are introduced simultaneously, especially in epidemic/pandemic situations. To more adequately predict future possible scenarios, multiple variable testing is needed.

In summary, hospitals must prepare for future scenarios and make well-developed guesses despite

lack of available evidence-based data. For this, SD modelling may assist decision-makers when planning preventive measures in the dynamic field of infectious diseases transmission.

7.9 PREVENTION AND CONTROL OF INFLUENZA VIRUS TRANSMISSION

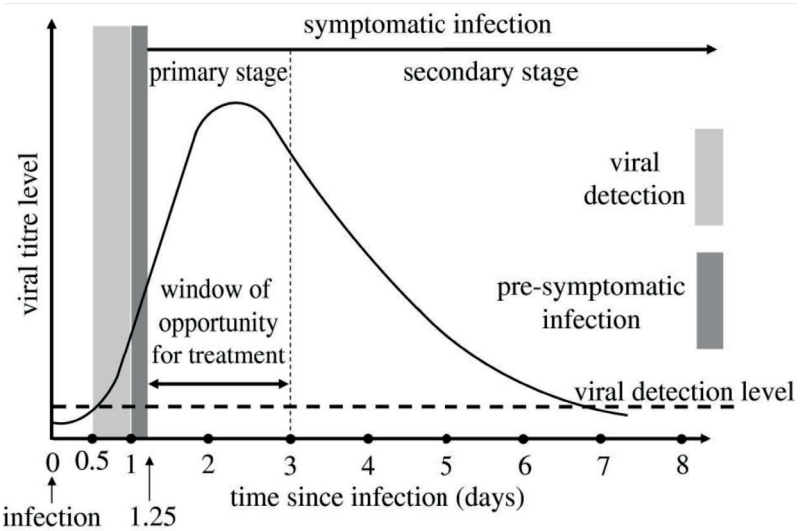
The chain of infection forms the basis of understanding transmission dynamics. It is described by CDC as 'an agent leaves its reservoir or host through a portal of exit, is conveyed by some mode of transmission, and enters through a portal of entry to infect a susceptible host'[149]. This illustrates the difficulties in presenting high grade evidence regarding transmission, as all the variables above need to be taken into consideration.

Viral properties for agents included in this thesis (HRV and influenza) have been discussed in previous sections. Remaining variables in the chain of infection for influenza are discussed separately below.

7.9.1 Reservoirs/Hosts

The main reservoir for influenza virus is the respiratory tract. Viral load in NPS peaks in median two days after symptom onset in experimentally infected volunteers [150] and is followed by a rapid decline over five days [151]. A schematic diagram of the viral dynamics of natural InfA infection is presented in Figure 17. Prolonged shedding has frequently been described in immunocompromised individuals [152, 153].

Figure 17: Dynamics of influenza A infection.



Emergence of drug resistance: implications for antiviral control of pandemic influenza. Murray E et al. Proceedings of Royals Society B Published 22 July 2007.DOI: 10.1098/rspb.2007.0422

Individuals infected by influenza are not equally infectious. In Paper II-III the median Ct value was 23 and 25 respectively, which suggest a high viral load among the hospital patient populations included in our studies. Clinically mild and even asymptomatic influenza infections may occur. A recent systematic review reported a pooled mean at 16% of the confirmed infections identified in a prospective community-based studies as being asymptomatic [154]. It remains however unclear to what extent these cases account for further transmission [155-157].

In Paper II-III, no cases were asymptomatic as they met the criteria for ILI/ARI and were tested at the discretion of the on treating physician. It is possible that asymptomatic or unrecognized symptomatic patients or HCWs might have contributed to transmission. Interviewing the fifteen HCWs who reported sick leave in Paper II (whereof 5/15 at the peak day of the epidemic curve) perhaps may have added useful information regarding a common source, although self-reporting of symptoms should be interpreted with care. Among HCWs working with influenza patients, attack rates have been described to range between 11-59% [158]. It is not unusual that HCWs continues to work when ill [159, 160].

Definitions of which symptoms are required for influenza case definitions may vary greatly [161], see Table 3. It has been suggested that only 50% to 79% of adults with confirmed influenza meet the ILI criteria [162]. If fever is required, the number of 'asymptomatic influenza infections' may be high, especially among the elderly [163]. A lack of fever has been reported among more than 50% of cases of HCWs with confirmed influenza [164].

Table 3: Influenza case definitions used in surveillance.

Definition	Type	Sudden onset	General symptoms	Respiratory symptoms
ECDC	ILI	Yes	At least one among: fever, feverishness, headache, malaise, myalgia	At least one among: cough, sore throata, shortness of breath
WHO	ILI	No	Fever ≥ 38 °C with onset within the last 10 days	Cough
CDC	ILI	Yes	Fever ≥ 100° F (37.8 °C)b Absence of a known cause other than influenza	At least one among: cough, sore throata
GROG	ARI	Yes	At least one among: fever ≥ 38 °C, headache, weakness, myalgia, chills	At least one among: cough, coryza, bronchitis, pharyngitis, shortness of breath, expectoration

ARI: Acute respiratory illness; CDC: Centers for Disease Control and Prevention; ECDC: European Centre for Disease Prevention and Control; GROG: Groupes Régionaux d'Observation de la Grippe; ILI: influenza-like illness; WHO: World Health Organization. a The sore throat symptom is not collected in the GROG network. For the purpose of this work, the variable was replaced by pharyngitis diagnosis. b Fever is defined in the GROG network as a temperature fever ≥ 100.4°F (38.0 °C). For the purpose of this work, fever ≥ 100° F (37.8 °C) was replaced by fever ≥ 100.4°F (38.0 °C).

Performance of influenza case definitions for influenza community surveillance: based on the French influenza surveillance network GROG, 2009-2014.Casalegno et al. Euro Surveill. 2017;22(14): pii=30504. https://doi.org/10.2807/1560-7917.ES.2017.22.14.30504 Received: 20 Nov 2015; Accepted: 14 Dec 2016 Creative Commons Attribution 4.0 International License.

7.9.2 Portal of exit, mode of transmission and portal of entry

The respiratory tract is the portal of exit and entry. Although much debated, it is generally believed that influenza transmission occurs mostly at a close range (by contact or droplets) and to a lesser extent by aerosols at greater distances [165]. It is important to distinguish influenza from pathogens which are predominantly airborne (e.g. measles, tuberculosis and varicella).

The potential for aerosol transmission for influenza should be regarded as much more dependent on various host, viral and environmental factors [9]. In Paper II, 7/20 cases supported in-ward transmission despite lack of evidence of close contact. Likewise, for Paper III, in two cases from two different wards a close relationship was found. Unrecognized links or aerosol transmission over longer distances might explain these cases. Future studies including WGS of larger samples from hospital populations have the potential to unravel chains of cryptogenic transmission.

Several studies have shown a wide variation in the viral load expelled by patients. When influenza shedding was evaluated in 61 patients, the highest emitters shed up to 32 times more virus compared to the others [166]. A study of 47 students found 81% cases positive for influenza RNA in cough aerosols with 65% of the particles at size <4 μ meter (thus possible to inhale). Moreover, particles expelled by coughing in influenza patients ranged from as low as 900 to 308 600/cough [167]. There are vast discrepancies on the number particles reported to be expelled during certain activities (e.g. by coughing, sneezing or talking). The differences in numbers are illustrated by 36 per 100 spoken words compared with 40 000 particles per sneeze according to Fernstrom et al [168]. Symptom severity scoring might be helpful in estimating infectivity in future prospective investigations but was not possible to convey in our studies.

The potential for aerosol transmission may be underestimated, especially as it is reported to be more efficient [50]. Another consideration reported in animal studies is that different strains may vary in their capacity for aerosol transmission [169]. While influenza also may be transmitted by indirect contact, it is impossible to determine the level of importance for each mode of transmission when working in close contact with patients. Studies of experimental infections (i.e. when healthy volunteers are infected with defined doses) may differ compared with normal exposure.

Evidence exist for barrier precautions and hand hygiene but remains poorly quantified [170, 171]. Respirators have not been shown superior compared with masks in preventing laboratory-confirmed influenza in a randomized control trial [172]. Experimental studies of mask efficacy supporting increased filtering capacity of influenza virus for respirators compared with masks in volunteers [173] may not translate into effectiveness in preventing infection.

Moreover, the existence of a policy does not equal adherence. Compliance with hand hygiene guidelines has been reported to be as low as 31-66% [174, 175]. Observation by trained observers remains the gold standard for measuring compliance [176], although new techniques are in the pipeline [177]. In our studies, adherence to control measures suggested for influenza patients was unfortunately not possible to evaluate.

7.9.3 Host susceptibility

Pre-existing immunity for influenza differs greatly among populations, and are influenced by factors such as age, sex, and innate immunity. It is

generally believed that multiple immune responses decline by age and thereby reduces the efficacy of influenza vaccination in the elderly [178]. Apart from differences in preexisting immunity, antiviral prophylaxis may offer protection, although a review by Cochrane found a 'modest effect' on prevention of symptomatic influenza in individuals [145]. Support for prophylactic use have been reported in terms of reduced rates of household transmission and shortening of outbreak durations in long-term care facilities [179]. Large, community-based studies on prophylactic use have yet to be performed.

In order to protect patients, vaccination of HCWs likely offers some indirect protection for risk groups although high-level evidence is lacking. Vaccination policies should be combined with work toward reducing presenteeism [180]. In Paper II, all HCWs reported sick during the outbreak were non-vaccinated but unfortunately no data regarding staff vaccination were available for Paper III.

7.9.4 Risk assessment

The risks for patients may be direct or indirect and depending on situation, setting, and population. Findings from studies conducted in long-term care facilities may not apply for acute-care with substantially higher patient throughput and shorter length-of-stay. Nursing homes likely have more stable patient and staff populations. Mortality rate for influenza in acute-care facilities and geriatric hospitals has been reported to be 16%, whereas in more vulnerable populations units it can be 33-60% [181-183]. Antiviral treatment is generally considered as safe, and since there are limited treatment options they remain widely recommended. In Paper III, the share of InfA patients treated with antivirals were 53% and 62% (non-HCAI and HCAI cases) which is much lower compared with a recent report from Australia [184].

Risk assessment including indirect consequences for patients and HCWs not directly involved in influenza transmission also need to be considered by the hospital management, if resources need to be allocated from other areas in order to control outbreaks.

7.9.5 Outbreak analysis

Hospital influenza outbreaks are likely substantially underreported [158] and are not well defined. Commonly at least two symptomatic patients within a 48-72 h period with a minimum of one laboratory confirmed case is used [92]. HCWs may facilitate transmission to patients and co-workers [185]. Early recognition is important for outbreak control and due to the broad clinical presentation [66], symptoms of 'suspected influenza' need to be clearly defined. The index case should not be confused with primary case [134]. The time of symptom on-set may be the only clue to estimate the point of time when the infection was acquired. Although often considered as common knowledge, statements of incubation time are often imprecise, unsourced and based on limited evidence [57].

The quality of research regarding hospital epidemiology often have major methodological weaknesses [144, 186]. Details regarding participants, settings, interventions, timing and potential confounders may be missing. Detailed contact tracing generally works well for stemming outbreaks of low-prevalence diseases, but effectiveness is limited for large outbreaks [187]. In 2007, the ORION statement was published, with "Guidance for transparent reporting of outbreak reports and intervention studies of nosocomial infection" [188]. Although more than ten years has passed, a large proportion of nosocomial outbreak reports do not provide basic information of the event. Reliable evidence-based data combined with experience may improve learning from previous outbreak experiences, but this goal

can only be achieved if critical data are reported [189].

Since the ORION statement was published, a rapid progress in sequencing technology has occurred which allow for earlier detection, uncovering of linked infections [190] and more precise investigation of outbreaks [191, 192]. For influenza, WGS offer superior resolution for molecular epidemiology compared to single segment analysis [193]. In a recent report from U.K, WGS data confirmed nosocomial transmission for approximately 16% of cases [194]. Equally to the impact of DNA-techniques on criminology, outbreak investigations need to include and integrate laboratory data with epidemiologic data to obtain full value [195, 196].

7.9.6 Concluding remarks

Epidemiological understanding of influenza transmission in healthcare settings remains incomplete [144]. Modelling studies may facilitate the understanding of complex processes and have the advantage of being cost-effective and ethically feasible. Although the risks for healthcare-associated influenza infections cannot be eliminated, there is still a duty to control transmission at an acceptable level. Emphasizing on HCW immunization, or any other single measure, is not enough on its own.

Surveillance must be adjusted to the needs of the facility and performed in a methodical and efficient manner. Laboratory testing may during some circumstances be performed by other implications than benefits for the individual patient [66]. With increasing demand for public reporting, the importance of standardized definitions and approaches for surveillance and outbreak detections cannot be overemphasized.

In situations where there is a lack of natural immunity, vaccination and therapy, no other measure than social distancing and supportive treatment remain. This is currently clearly illustrated by the mitigation measures we are forced to use for the Covid-19 pandemic. For hospital transmission of influenza, we are still lucky to have a broader set of control measures, elegantly summarized in the article by Vanhems et al [142].

CONCLUSIONS

Epidemiology of viral
respiratory infections
with focus on in-hospital
influenza transmission

MARTINA SANSONE

8 CONCLUSIONS

- Locally circulating HRV strains represent several types and seem to reflect that these infections are highly globalized. The existence of simultaneous or successive epidemics with different HRV types, in combination with the ability of each type to remain in the local population over extended periods of time, may contribute to explain the high rate of HRV infections.

- Influenza B virus may spread efficiently within an acute-care hospital, and advanced molecular methods may facilitate assessment of the source and extent of an outbreak.

- In-ward transmission of Influenza A occurs frequently, and healthcare-associated influenza may have a severe outcome. Whole-genome sequencing can be used for outbreak investigations and evaluation of preventive measures.

- System dynamic modelling may be a valuable tool to illustrate in-hospital transmission of influenza. According to our model, antiviral prophylaxis to exposed patients seem to be the most effective way to control in-hospital transmission.

FUTURE PERSPECTIVES

Epidemiology of viral respiratory infections with focus on in-hospital influenza transmission

MARTINA SANSONE

9 FUTURE PERSPECTIVES

Although this thesis added some knowledge in the field of epidemiology and transmission for HRV and influenza, there is a wide range of unanswered questions along with great possibilities for future research. I will finish by sharing some of my predictions for the future below.

Viruses will continue to challenge humans. Some viral infections will be defeated, but new ones will arise. Climate change, travelling patterns (illustrated in Figure 19) and urbanization create new environments which may pave the way for previously unknown and new diseases. This manifested today, when SARS-CoV-2 rapidly and dramatically has changed the lives for millions of people. We can directly observe how a respiratory virus efficiently may spread in absence of pre-existing immunity, vaccine or treatment options.

While the world has a high interest in viruses, intersectional cooperation within virology, medicine, public health, epidemiology, computer science and operation's research are needed and will hopefully join forces to synthesize information and increase public knowledge.

Based on experiences from SARS-CoV-2, we might in the future need to pay more attention on the share of unrecognized/undiagnosed cases in a society and include them in assumptions regarding transmission. Just because things not yet are discovered, they still may exist.

Previously known merely as a large group of diseases with similar clinical presentation (ARI/RTI or ILI), PCR increased our understanding of viral infectious diseases. With the advances in molecular epidemiology, new insights will arise and WGS is next in line to revolutionize outbreak analysis and public health surveillance.

The HCAI definition needs to be completed with criteria for a hospital-acquired infection, preferably defined as possible, probable or proven. Hopefully legal and insurance controversies won't affect the much-desired need for a standardization.

WGS will add significant value for infection prevention and control and public health in order to confirm or uncover transmission links. Laboratory and epidemiologic data have previously often been stored separately, but this data need to be integrated in order to gain full value and direct measures to where it has most impact.

Point-of-care PCR testing for respiratory viruses are already increasingly being used at emergency departments. Easy access combined with shortened answering times will enable control measures upon admission, co-horting of patients and early treatment initiation.

New possibilities to self-sampling and at home diagnostics will evolve. Though access to laboratory diagnostics can be easily arranged, increased demand of interpreting the results will arise.

A universal vaccine replacing the annual seasonal influenza vaccine will hopefully be developed. By targeting influenza's highly conserved protein regions, it may be possible to induce cross-protective immunity.
In the work against antimicrobial resistance, viral infections will be included. By diagnosing viral RTIs and reducing HCAIs caused by respiratory viruses, less antibiotics will be prescribed.

How shall we efficiently plan and use our healthcare resources in the future? There is a need to create a dialogue with healthcare providers and resource management on which methods to choose in controlling transmissible infections. Sweden has the lowest number of hospital beds within the EU [197] and Kungälv hospital, described in this thesis, has the highest occupancy rate in the region. Overcrowded hospital wards, lack of staff and multiple transfer of patients within the hospital may increase the number of exposed patients when an outbreak occurs. In order to save both resources and lives in the future, it is time to change the focus from writing policies to real-world outcomes.

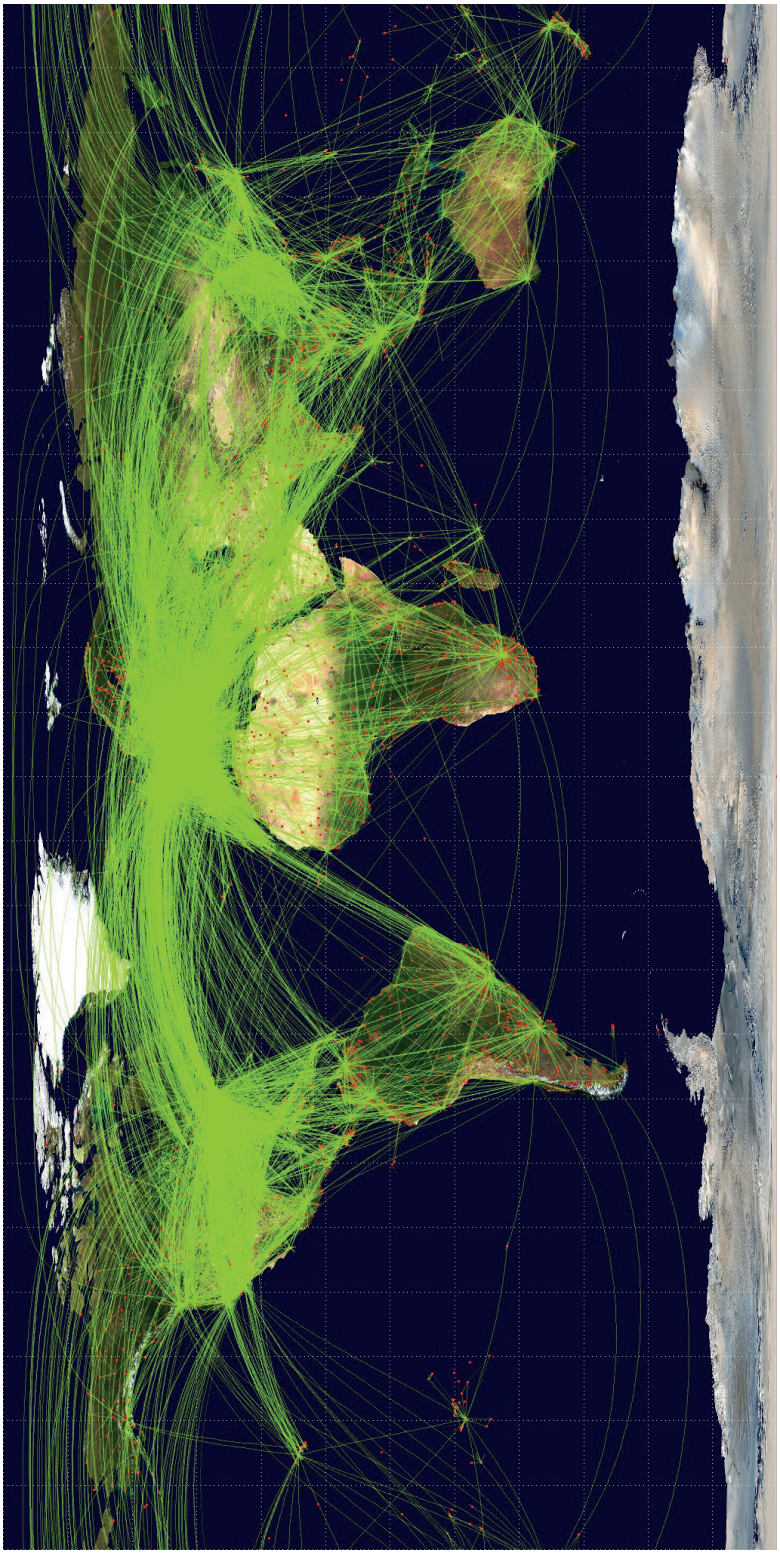


Figure 19: World airline route-map before the Covid-19 pandemic.

ACKNOW-LEDGEMENT

Epidemiology of viral respiratory infections with focus on in-hospital influenza transmission

MARTINA SANSONE

ACKNOWLEDGEMENT

Johan Westin, main supervisor. For great friendship, gentle handling of my ups and downs and for numerous laughs along the way. You create an inspiring and supportive research atmosphere.

Lars-Magnus Andersson, co-supervisor. For sharpness as well as enthusiasm when most needed. For making me want to do my best and for teaching me the importance of strategic thinking.

Rickard Nordén, co-supervisor. For accepting me as one of your PhD students without hesitation. You have been this thesis knight in shining armor. Although you always scare me, don´t stop dropping by.

Maria Andersson, co-author. For skillful laboratory work and for teaching me some of the really difficult things. Your smile is a ray of sunshine at Guldhedsgatan.

Magnus Lindh, co-author. For making Clinical Virology a true center of excellence. For generously sharing your time and expertise as well as deeply hidden Excel files.

Lars Gustavsson, co-author. For altruistic guidance in various fields, from SPSS to nice restaurants. You are the backbone and the future of the "Goodenough Group".

Sigvard Olofsson and **Lars Bohlin,** co-authors. For your positivity and valuable contribution.

Åsa Wiman, Mia Brytting and **Maria Lind Karlberg, Public Health Agency of Sweden,** co-authors. For sharing your expertise and for excellent collaboration.

Paul Holmström and **Stefan Hallberg,** co-authors. For your open mind, curiosity and ambition to improve healthcare by system dynamic modelling.

Nicklas Sundell, Thomas Beck-Friis and **Robin Brittain-Long.** Research is easy with you in the team.

Former and current head of departments: **Rune Wejstål, Lars-Magnus Andersson, Christina Welinder-Olsson, Rickard Nordén** and **Carin Gebäck.** For giving me the opportunity to do research.

Friends and current colleagues at the **Infection Control Unit.** For generously introducing me to new fields and for having patience with my impatience. Special thanks to **Kerstin, Osk, Roseli** and **Lotta** for your assistance and interest in influenza control.

Friends and former colleagues at **IKÖ**. I miss you all.

Anna Lindblom, partner-in-crime at Clinical Microbiology. Congratulations to both of us. Now is the time for champagne and new beginnings!

Sofia Myhrman. For bringing sparkle into my daily life and for instantly volunteering to be toastmaster.

Leif Dotevall and **Thomas Wahlberg,** SME. For sharing my interest in outbreak investigations and for great collaboration in daily work.

Katarina Lindström Johansson and the rest of the staff at the detection unit, **Clinical Virology.** For always being helpful in spite of an endless flow of PhD students.

Olle Westin. For helping me out with tiresome paperwork.

My friends from the **volleyball and beach volleyball field.** You know that my true habitat is far from a desk in front of a computer.

My new friends, old friends and **very old friends.** I look forward to inspiring talks and more time to cook for you in the future.

My sister **Ulrika** and my brother **Niclas.** For caring for me and treating me as an equal despite my younger age.

My parents **Hans** and **Hjördis.** For giving me plenty of freedom and always trust my judgement and my capacity. Your strategy of never telling me to do my homework seem to have worked...

My husband **Mikael.** For being the handsome rock beside me since nearly 25 years. Ti amo.

My children **Saga** and **Elias.** For unconditional love and being amazing just the way you are.

REFERENCES

REFERENCES

1. McNeill WH. Plagues and peoples. New York: Anchor Books, 1989.

2. Alsved M, Fraenkel CJ, Bohgard M, et al. Sources of Airborne Norovirus in Hospital Outbreaks. Clin Infect Dis 2019.

3. Nenonen NP, Hannoun C, Svensson L, et al. Norovirus GII.4 detection in environmental samples from patient rooms during nosocomial outbreaks. J Clin Microbiol 2014; 52(7): 2352-8.

4. Best M, Neuhauser D. Ignaz Semmelweis and the birth of infection control. Qual Saf Health Care 2004; 13(3): 233-4.

5. Webb JL, Jr. The historical epidemiology of global disease challenges. Lancet 2015; 385(9965): 322-3.

6. CDC Library (U.S.), Center for Disease Control. CDC Library serial holdings. Atlanta, Ga.: Center for Disease Control.

7. Pappas DE, Hendley JO, Hayden FG, Winther B. Symptom profile of common colds in school-aged children. Pediatr Infect Dis J 2008; 27(1): 8-11.

8. Collaborators GBDCoD. Global, regional, and national age-sex specific mortality for 264 causes of death, 1980-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet 2017; 390(10100): 1151-210.

9. Tellier R, Li Y, Cowling BJ, Tang JW. Recognition of aerosol transmission of infectious agents: a commentary. BMC Infect Dis 2019; 19(1): 101.

10. Jacobs SE, Lamson DM, St George K, Walsh TJ. Human rhinoviruses. Clin Microbiol Rev 2013; 26(1): 135-62.

11. Fendrick AM. Viral respiratory infections due to rhinoviruses: current knowledge, new developments. Am J Ther 2003; 10(3): 193-202.

12. Bizzintino J, Lee WM, Laing IA, et al. Association between human rhinovirus C and severity of acute asthma in children. Eur Respir J 2011; 37(5): 1037-42.

13. Heymann PW, Carper HT, Murphy DD, et al. Viral infections in relation to age, atopy, and season of admission among children hospitalized for wheezing. J Allergy Clin Immunol 2004; 114(2): 239-47.

14. Kurai D, Saraya T, Ishii H, Takizawa H. Virus-induced exacerbations in asthma and COPD. Front Microbiol 2013; 4: 293.

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

16. Cassini A, Plachouras D, Eckmanns T, et al. Burden of Six Healthcare-Associated Infections on European Population Health: Estimating Incidence-Based Disability-Adjusted Life Years through a Population Prevalence-Based Modelling Study. PLoS Med 2016; 13(10): e1002150.

17. Bochkov YA, Palmenberg AC, Lee WM, et al. Molecular modeling, organ culture and reverse genetics for a newly identified human rhinovirus C. Nat Med 2011; 17(5): 627-32.

18. Lau SK, Yip CC, Lin AW, et al. Clinical and molecular epidemiology of human rhinovirus C in children and adults in Hong Kong reveals a possible distinct human rhinovirus C subgroup. J Infect Dis 2009; 200(7): 1096-103.

19. Miller EK, Edwards KM, Weinberg GA, et al. A novel group of rhinoviruses is associated with asthma hospitalizations. J Allergy Clin Immunol 2009; 123(1): 98-104 e1.

20. Piralla A, Rovida F, Campanini G, et al. Clinical severity and molecular typing of human rhinovirus C strains during a fall outbreak affecting hospitalized patients. J Clin Virol 2009; 45(4): 311-7.

21. Iwane MK, Prill MM, Lu X, et al. Human rhinovirus species associated with hospitalizations for acute respiratory illness in young US children. J Infect Dis 2011; 204(11): 1702-10.

22. Xiang Z, Gonzalez R, Xie Z, et al. Human rhinovirus C infections mirror those of human rhinovirus A in children with community-acquired pneumonia. J Clin Virol 2010; 49(2): 94-9.

23. Hendley JO, Wenzel RP, Gwaltney JM, Jr. Transmission of rhinovirus colds by self-inoculation. N Engl J Med 1973; 288(26): 1361-4.

24. Winther B, McCue K, Ashe K, Rubino J, Hendley JO. Rhinovirus contamination of surfaces in homes of adults with natural colds: transfer of virus to fingertips during normal daily activities. J Med Virol 2011; 83(5): 906-9.

25. Sandora TJ, Shih MC, Goldmann DA. Reducing absenteeism from gastrointestinal and respiratory illness in elementary school students: a randomized, controlled trial of an infection-control intervention. Pediatrics 2008; 121(6): e1555-62.

26. Winther B. Rhinovirus infections in the upper airway. Proc Am Thorac Soc 2011; 8(1): 79-89.

27. Brownlee JW, Turner RB. New developments in the epidemiology and clinical spectrum of rhinovirus infections. Curr Opin Pediatr 2008; 20(1): 67-71.

28. Lessler J, Brookmeyer R, Reich NG, Nelson KE, Cummings DA, Perl TM. Identifying the probable timing and setting of respiratory virus infections. Infect Control Hosp Epidemiol 2010; 31(8): 809-15.

29. Kennedy JL, Turner RB, Braciale T, Heymann PW, Borish L. Pathogenesis of rhinovirus infection. Curr Opin Virol 2012; 2(3): 287-93.

30. Midulla F, Pierangeli A, Cangiano G, et al. Rhinovirus bronchiolitis and recurrent wheezing: 1-year follow-up. Eur Respir J 2012; 39(2): 396-402.

31. Bellei N, Carraro E, Perosa A, Watanabe A, Arruda E, Granato C. Acute respiratory infection and influenza-like illness viral etiologies in Brazilian adults. J Med Virol 2008; 80(10): 1824-7.

32. Ison MG. Respiratory viral infections in transplant recipients. Antivir Ther 2007; 12(4 Pt B): 627-38.

33. Gerna G, Piralla A, Rovida F, et al. Correlation of rhinovirus load in the respiratory tract and clinical symptoms in hospitalized immunocompetent and immunocompromised patients. J Med Virol 2009; 81(8): 1498-507.

34. Peltola V, Waris M, Kainulainen L, Kero J, Ruuskanen O. Virus shedding after human rhinovirus infection in children, adults and patients with hypogammaglobulinaemia. Clin Microbiol Infect 2013; 19(7): E322-7.

35. Kaiser L, Aubert JD, Pache JC, et al. Chronic rhinoviral infection in lung transplant recipients. Am J Respir Crit Care Med 2006; 174(12): 1392-9.

36. Pathak AK, Adams RH, Shah NC, Gustin KE. Persistent human rhinovirus type C infection of the lower respiratory tract in a pediatric cord blood transplant recipient. Bone Marrow Transplant 2013; 48(5): 747-8.

37. Reis J, Shaman J. Simulation of four respiratory viruses and inference of epidemiological parameters. Infect Dis Model 2018; 3: 23-34.

38. Peltola V, Waris M, Osterback R, Susi P, Ruuskanen O, Hyypia T. Rhinovirus transmission within families with children: incidence of symptomatic and asymptomatic infections. J Infect Dis 2008; 197(3): 382-9.

39. Turner RB. Rhinovirus infection of human embryonic lung fibroblasts induces the production of a chemoattractant for polymorphonuclear leukocytes. J Infect Dis 1988; 157(2): 346-50.

40. Barclay WS, al-Nakib W, Higgins PG, Tyrrell DA. The time course of the humoral immune response to rhinovirus infection. Epidemiol Infect 1989; 103(3): 659-69.

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

42. Shaw MW, Arden NH, Maassab HF. New aspects of influenza viruses. Clin Microbiol Rev 1992; 5(1): 74-92.

43. Rota PA, Wallis TR, Harmon MW, Rota JS, Kendal AP, Nerome K. Cocirculation of two distinct evolutionary lineages of influenza type B virus since 1983. Virology 1990; 175(1): 59-68.

44. Nobusawa E, Sato K. Comparison of the mutation rates of human influenza A and B viruses. J Virol 2006; 80(7): 3675-8.

45. Yang Y, Halloran ME, Sugimoto JD, Longini IM, Jr. Detecting human-to-human transmission of avian influenza A (H5N1). Emerg Infect Dis 2007; 13(9): 1348-53.

46. Ungchusak K, Auewarakul P, Dowell SF, et al. Probable person-to-person transmission of avian influenza A (H5N1). N Engl J Med 2005; 352(4): 333-40.

47. Edenborough KM, Lowther S, Laurie K, et al. Predicting Disease Severity and Viral Spread of H5N1 Influenza Virus in Ferrets in the Context of Natural Exposure Routes. J Virol 2016; 90(4): 1888-97.

48. Marois I, Cloutier A, Garneau E, Richter MV. Initial infectious dose dictates the innate, adaptive, and memory responses to influenza in the respiratory tract. J Leukoc Biol 2012; 92(1): 107-21.

49. Memoli MJ, Czajkowski L, Reed S, et al. Validation of the wild-type influenza A human challenge model H1N1pdMIST: an A(H1N1)pdm09 dose-finding investigational new drug study. Clin Infect Dis 2015; 60(5): 693-702.

50. Alford RH, Kasel JA, Gerone PJ, Knight V. Human influenza resulting from aerosol inhalation. Proc Soc Exp Biol Med 1966; 122(3): 800-4.

51. Treanor JJ. Influenza virus. 6 ed: Elsevier Churchill Livingstone; 2005., 2005.

52. Bean B, Moore BM, Sterner B, Peterson LR, Gerding DN, Balfour HH, Jr. Survival of influenza viruses on environmental surfaces. J Infect Dis 1982; 146(1): 47-51.

53. Mukherjee DV, Cohen B, Bovino ME, Desai S, Whittier S, Larson EL. Survival of influenza virus on hands and fomites in community and laboratory settings. Am J Infect Control 2012; 40(7): 590-4.

54. Greatorex JS, Digard P, Curran MD, et al. Survival of influenza A(H1N1) on materials found in households: implications for infection control. PLoS One 2011; 6(11): e27932.

55. Oxford J, Berezin EN, Courvalin P, et al. The survival of influenza A(H1N1)pdm09 virus on 4 household surfaces. Am J Infect Control 2014; 42(4): 423-5.

56. Ma W, Kahn RE, Richt JA. The pig as a mixing vessel for influenza viruses: Human and veterinary implications. J Mol Genet Med 2008; 3(1): 158-66.

57. Lessler J, Reich NG, Brookmeyer R, Perl TM, Nelson KE, Cummings DA. Incubation periods of acute respiratory viral infections: a systematic review. Lancet Infect Dis 2009; 9(5): 291-300.

58. Nicholson KG. Clinical features of influenza. Semin Respir Infect 1992; 7(1): 26-37.

59. Wright PF, Bryant JD, Karzon DT. Comparison of influenza B/Hong Kong virus infections among infants, children, and young adults. J Infect Dis 1980; 141(4): 430-5.

60. Kalil AC, Thomas PG. Influenza virus-related critical illness: pathophysiology and epidemiology. Crit Care 2019; 23(1): 258.

61. Cox NJ. Prevention and control of influenza. Lancet 1999; 354 Suppl: SIV30.

62. Long P, Probe. Principles and practice of pediatric infectious diseases: Elsevier, 2012.

63. Gill JR, Sheng ZM, Ely SF, et al. Pulmonary pathologic findings of fatal 2009 pandemic influenza A/H1N1 viral infections. Arch Pathol Lab Med 2010; 134(2): 235-43.

64. Shieh WJ, Blau DM, Denison AM, et al. 2009 pandemic influenza A (H1N1): pathology and pathogenesis of 100 fatal cases in the United States. Am J Pathol 2010; 177(1): 166-75.

65. Sellers SA, Hagan RS, Hayden FG, Fischer WA, 2nd. The hidden burden of influenza: A review of the extra-pulmonary complications of influenza infection. Influenza Other Respir Viruses 2017; 11(5): 372-93.

66. Chow EJ, Rolfes MA, O'Halloran A, et al. Respiratory and Nonrespiratory Diagnoses Associated With Influenza in Hospitalized Adults. JAMA Netw Open 2020; 3(3): e201323.

67. Thompson WW, Shay DK, Weintraub E, et al. Mortality associated with influenza and respiratory syncytial virus in the United States. JAMA 2003; 289(2): 179-86.

68. Thompson WW, Shay DK, Weintraub E, et al. Influenza-associated hospitalizations in the United States. JAMA 2004; 292(11): 1333-40.

69. Thompson WW, Moore MR, Weintraub E, et al. Estimating influenza-associated deaths in the United States. Am J Public Health 2009; 99 Suppl 2: S225-30.

70. FoHM. Public Health Agency of Sweden -Current Influenza report. Available at: https://www.folkhalsomyndigheten.se/publicerat-material/publikationsarkiv/i/influenza-in-sweden-2016-2017-season/.

71. Caini S, Kroneman M, Wiegers T, El Guerche-Seblain C, Paget J. Clinical characteristics and severity of influenza infections by virus type, subtype, and lineage: A systematic literature review. Influenza Other Respir Viruses 2018; 12(6): 780-92.

72. Mertz D, Kim TH, Johnstone J, et al. Populations at risk for severe or complicated influenza illness: systematic review and meta-analysis. BMJ 2013; 347: f5061.

73. Sullivan S. Challenges in reducing influenza-associated mortality. Lancet 2018; 391(10127): 1242-4.

74. Iuliano AD, Roguski KM, Chang HH, et al. Estimates of global seasonal influenza-associated respiratory mortality: a modelling study. Lancet 2018; 391(10127): 1285-300.

75. Walker TA, Waite B, Thompson MG, et al. Risk of Severe Influenza Among Adults With Chronic Medical Conditions. J Infect Dis 2020; 221(2): 183-90.

76. Czaja CA, Miller L, Alden N, et al. Age-Related Differences in Hospitalization Rates, Clinical Presentation, and Outcomes Among Older Adults Hospitalized With Influenza-U.S. Influenza Hospitalization Surveillance Network (FluSurv-NET). Open Forum Infect Dis 2019; 6(7).

77. FoHm. Public Health Agency of Sweden - Seasonal Flu Report.

78. Francis T, Jr., Davenport FM, Hennessy AV. A serological recapitulation of human infection with different strains of influenza virus. Trans Assoc Am Physicians 1953; 66: 231-9.

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

80. Biggerstaff M, Cauchemez S, Reed C, Gambhir M, Finelli L. Estimates of the reproduction number for seasonal, pandemic, and zoonotic influenza: a systematic review of the literature. BMC Infect Dis 2014; 14: 480.

81. Lofgren E, Fefferman NH, Naumov YN, Gorski J, Naumova EN. Influenza seasonality: underlying causes and modeling theories. J Virol 2007; 81(11): 5429-36.

82. Fleming DM, Zambon M, Bartelds AI, de Jong JC. The duration and magnitude of influenza epidemics: a study of surveillance data from sentinel general practices in England, Wales and the Netherlands. Eur J Epidemiol 1999; 15(5): 467-73.

83. Souza LS, Ramos EA, Carvalho FM, et al. Viral respiratory infections in young children attending day care in urban Northeast Brazil. Pediatr Pulmonol 2003; 35(3): 184-91.

84. Lowen AC, Steel J. Roles of humidity and temperature in shaping influenza seasonality. J Virol 2014; 88(14): 7692-5.

85. Sundell N, Andersson LM, Brittain-Long R, Lindh M, Westin J. A four year seasonal survey of the relationship between outdoor climate and epidemiology of viral respiratory tract infections in a temperate climate. J Clin Virol 2016; 84: 59-63.

86. Tamerius JD, Shaman J, Alonso WJ, et al. Environmental predictors of seasonal influenza epidemics across temperate and tropical climates. PLoS Pathog 2013; 9(3): e1003194.

87. Gasparini R, Amicizia D, Lai PL, Bragazzi NL, Panatto D. Compounds with anti-influenza activity: present and future of strategies for the optimal treatment and management of influenza. Part II: Future compounds against influenza virus. J Prev Med Hyg 2014; 55(4): 109-29.

88. Gasparini R, Amicizia D, Lai PL, Bragazzi NL, Panatto D. Compounds with anti-influenza activity: present and future of strategies for the optimal treatment and management of influenza. Part I: Influenza life-cycle and currently available drugs. J Prev Med Hyg 2014; 55(3): 69-85.

89. Houser K, Subbarao K. Influenza vaccines: challenges and solutions. Cell Host Microbe 2015; 17(3): 295-300.

90. Sullivan SG, Chilver MB, Higgins G, Cheng AC, Stocks NP. Influenza vaccine effectiveness in Australia: results from the Australian Sentinel Practices Research Network. Med J Aust 2014; 201(2): 109-11.

91. Andersson ME, Olofsson S, Lindh M. Comparison of the FilmArray assay and in-house real-time PCR for detection of respiratory infection. Scand J Infect Dis 2014; 46(12): 897-901.

92. Harper SA, Bradley JS, Englund JA, et al. Seasonal influenza in adults and children--diagnosis, treatment, chemoprophylaxis, and institutional outbreak management: clinical practice guidelines of the Infectious Diseases Society of America. Clin Infect Dis 2009; 48(8): 1003-32.

93. Meijer A, Rebelo-de-Andrade H, Correia V, et al. Global update on the susceptibility of human influenza viruses to neuraminidase inhibitors, 2012-2013. Antiviral Res 2014; 110: 31-41.

94. Dobson J, Whitley RJ, Pocock S, Monto AS. Oseltamivir treatment for influenza in adults: a meta-analysis of randomised controlled trials. Lancet 2015; 385(9979): 1729-37.

95. Haley RW, Quade D, Freeman HE, Bennett JV. The SENIC Project. Study on the efficacy of nosocomial infection control (SENIC Project). Summary of study design. Am J Epidemiol 1980; 111(5): 472-85.

96. Siegel JD, Rhinehart E, Jackson M, Chiarello L, Health Care Infection Control Practices Advisory C. 2007 Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Health Care Settings. Am J Infect Control 2007; 35(10 Suppl 2): S65-164.

97. WHO. The burden of health care-associated infection worldwide.

98. ECDC. Annual epidemiological report on communicable diseases, 2008.

99. Moralejo D, El Dib R, Prata RA, Barretti P, Correa I. Improving adherence to Standard Precautions for the control of health care-associated infections. Cochrane Database Syst Rev 2018; 2: CD010768.

100. Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. Am J Infect Control 2008; 36(5): 309-32.

101. Benet T, Regis C, Voirin N, et al. Influenza vaccination of healthcare workers in acute-care hospitals: a case-control study of its effect on hospital-acquired influenza among patients. BMC Infect Dis 2012; 12: 30.

102. Weber DJ, Rutala WA, Miller MB, Huslage K, Sickbert-Bennett E. Role of hospital surfaces in the transmission of emerging health care-associated pathogens: norovirus, Clostridium difficile, and Acinetobacter species. Am J Infect Control 2010; 38(5 Suppl 1): S25-33.

103. Otter JA, Yezli S, Salkeld JA, French GL. Evidence that contaminated surfaces contribute to the transmission of hospital pathogens and an overview of strategies to address contaminated surfaces in hospital settings. Am J Infect Control 2013; 41(5 Suppl): S6-11.

104. Sanger F, Brownlee GG, Barrell BG. A two-dimensional fractionation procedure for radioactive nucleotides. J Mol Biol 1965; 13(2): 373-98.

105. Margulies M, Egholm M, Altman WE, et al. Genome sequencing in microfabricated high-density picolitre reactors. Nature 2005; 437(7057): 376-80.

106. Bentley DR, Balasubramanian S, Swerdlow HP, et al. Accurate whole human genome sequencing using reversible terminator chemistry. Nature 2008; 456(7218): 53-9.

107. Voelkerding KV, Dames SA, Durtschi JD. Next-generation sequencing: from basic research to diagnostics. Clin Chem 2009; 55(4): 641-58.

108. Rothberg JM, Hinz W, Rearick TM, et al. An integrated semiconductor device enabling non-optical genome sequencing. Nature 2011; 475(7356): 348-52.

109. Stein LD. The case for cloud computing in genome informatics. Genome Biol 2010; 11(5): 207.

110. Eisenstein M. Oxford Nanopore announcement sets sequencing sector abuzz. Nat Biotechnol 2012; 30(4): 295-6.

111. Check Hayden E. Pint-sized DNA sequencer impresses first users. Nature 2015; 521(7550): 15-6.

112. Johnson SS, Zaikova E, Goerlitz DS, Bai Y, Tighe SW. Real-Time DNA Sequencing in the Antarctic Dry Valleys Using the Oxford Nanopore Sequencer. J Biomol Tech 2017; 28(1): 2-7.

113. Borges V, Pinheiro M, Pechirra P, Guiomar R, Gomes JP. INSaFLU: an automated open web-based bioinformatics suite "from-reads" for influenza whole-genome-sequencing-based surveillance. Genome Med 2018; 10(1): 46.

114. Revez J, Espinosa L, Albiger B, et al. Survey on the Use of Whole-Genome Sequencing for Infectious Diseases Surveillance: Rapid Expansion of European National Capacities, 2015-2016. Front Public Health 2017; 5: 347.

115. Goldstein EJ, Harvey WT, Wilkie GS, et al. Integrating patient and whole-genome sequencing data to provide insights into the epidemiology of seasonal influenza A(H3N2) viruses. Microb Genom 2018; 4(1).

116. Zuckerkandl E, Pauling L. Molecules as documents of evolutionary history. J Theor Biol 1965; 8(2): 357-66.

117. Gagarin A, Makarenkov V, Zentilli P. Using clustering techniques to improve hit selection in high-throughput screening. J Biomol Screen 2006; 11(8): 903-14.

118. Whelan S, Lio P, Goldman N. Molecular phylogenetics: state-of-the-art methods for looking into the past. Trends Genet 2001; 17(5): 262-72.

119. Yokoyama S, Felsenstein J. A model of kin selection for an altruistic trait considered as a quantitative character. Proc Natl Acad Sci U S A 1978; 75(1): 420-2.

120. Som A. Causes, consequences and solutions of phylogenetic incongruence. Brief Bioinform 2015; 16(3): 536-48.

121. Paszkiewicz K, Studholme DJ. De novo assembly of short sequence reads. Brief Bioinform 2010; 11(5): 457-72.

122. Miller JR, Koren S, Sutton G. Assembly algorithms for next-generation sequencing data. Genomics 2010; 95(6): 315-27.

123. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis 1987; 40(5): 373-83.

124. Simmonds P, McIntyre C, Savolainen-Kopra C, Tapparel C, Mackay IM, Hovi T. Proposals for the classification of human rhinovirus species C into genotypically assigned types. J Gen Virol 2010; 91(Pt 10): 2409-19.

125. Tapparel C, Cordey S, Van Belle S, et al. New molecular detection tools adapted to emerging rhinoviruses and enteroviruses. J Clin Microbiol 2009; 47(6): 1742-9.

126. Richter J, Nikolaou E, Panayiotou C, Tryfonos C, Koliou M, Christodoulou C. Molecular epidemiology of rhinoviruses in Cyprus over three consecutive seasons. Epidemiol Infect 2015; 143(9): 1876-83.

127. Daleno C, Piralla A, Scala A, Senatore L, Principi N, Esposito S. Phylogenetic analysis of human rhinovirus isolates collected from otherwise healthy children with community-acquired pneumonia during five successive years. PLoS One 2013; 8(11): e80614.

128. Andres C, Peremiquel-Trillas P, Gimferrer L, et al. Genetic diversity of rhinoviruses detected at a tertiary hospital in Catalonia (Spain) during the 2014-2017 seasons. Future Microbiol 2018; 13: 1565-73.

129. Henquell C, Mirand A, Deusebis AL, et al. Prospective genotyping of human rhinoviruses in children and adults during the winter of 2009-2010. J Clin Virol 2012; 53(4): 280-4.

130. Ajelli M, Merler S, Fumanelli L, et al. Spatiotemporal dynamics of the Ebola epidemic in Guinea and implications for vaccination and disease elimination: a computational modeling analysis. BMC Med 2016; 14(1): 130.

131. Ledford RM, Patel NR, Demenczuk TM, et al. VP1 sequencing of all human rhinovirus serotypes: insights into genus phylogeny and susceptibility to antiviral capsid-binding compounds. J Virol 2004; 78(7): 3663-74.

132. Snitkin ES, Zelazny AM, Thomas PJ, et al. Tracking a hospital outbreak of carbapenem-resistant Klebsiella pneumoniae with whole-genome sequencing. Sci Transl Med 2012; 4(148): 148ra16.

133. Pauly MD, Procario MC, Lauring AS. A novel twelve class fluctuation test reveals higher than expected mutation rates for influenza A viruses. Elife 2017; 6.

134. Giesecke J. Primary and index cases. Lancet 2014; 384(9959): 2024.

135. Cowling BJ, Ip DK, Fang VJ, et al. Aerosol transmission is an important mode of influenza A virus spread. Nat Commun 2013; 4: 1935.

136. Yan J, Grantham M, Pantelic J, et al. Infectious virus in exhaled breath of symptomatic seasonal influenza cases from a college community. Proc Natl Acad Sci U S A 2018; 115(5): 1081-6.

137. Rainwater-Lovett K, Chun K, Lessler J. Influenza outbreak control practices and the effectiveness of interventions in long-term care facilities: a systematic review. Influenza Other Respir Viruses 2014; 8(1): 74-82.

138. Song JY, Cheong HJ, Hwang IS, et al. Long-term immunogenicity of influenza vaccine among the elderly: Risk factors for poor immune response and persistence. Vaccine 2010; 28(23): 3929-35.

139. Young B, Zhao X, Cook AR, Parry CM, Wilder-Smith A, MC IC. Do antibody responses to the influenza vaccine persist year-round in the elderly? A systematic review and meta-analysis. Vaccine 2017; 35(2): 212-21.

140. Houlihan CF, Frampton D, Ferns RB, et al. Use of Whole-Genome Sequencing in the Investigation of a Nosocomial Influenza Virus Outbreak. J Infect Dis 2018; 218(9): 1485-9.

141. Houghton R, Ellis J, Galiano M, Clark TW, Wyllie S. Haemagglutinin and neuraminidase sequencing delineate nosocomial influenza outbreaks with accuracy equivalent to whole genome sequencing. J Infect 2017; 74(4): 377-84.

142. Vanhems P, Benet T, Munier-Marion E. Nosocomial influenza: encouraging insights and future challenges. Curr Opin Infect Dis 2016; 29(4): 366-72.

143. Jhung MA, D'Mello T, Perez A, et al. Hospital-onset influenza hospitalizations--United States, 2010-2011. Am J Infect Control 2014; 42(1): 7-11.

144. Voirin N, Barret B, Metzger MH, Vanhems P. Hospital-acquired influenza: a synthesis using the Outbreak Reports and Intervention Studies of Nosocomial Infection (ORION) statement. J Hosp Infect 2009; 71(1): 1-14.

145. Jefferson T, Jones MA, Doshi P, et al. Neuraminidase inhibitors for preventing and treating influenza in healthy adults and children. Cochrane Database Syst Rev 2014; (4): CD008965.

146. Okoli GN, Otete HE, Beck CR, Nguyen-Van-Tam JS. Use of neuraminidase inhibitors for rapid containment of influenza: a systematic review and meta-analysis of individual and household transmission studies. PLoS One 2014; 9(12): e113633.

147. Tsang TK, Cowling BJ, Fang VJ, et al. Influenza A Virus Shedding and Infectivity in Households. J Infect Dis 2015; 212(9): 1420-8.

148. Munier-Marion E, Benet T, Regis C, Lina B, Morfin F, Vanhems P. Hospitalization in double-occupancy rooms and the risk of hospital-acquired influenza: a prospective cohort study. Clin Microbiol Infect 2016; 22(5): 461 e7-9.

149. CDC. An Introduction to Applied Epidemiology and Biostatistics.

150. Carrat F, Vergu E, Ferguson NM, et al. Time lines of infection and disease in human influenza: a review of volunteer challenge studies. Am J Epidemiol 2008; 167(7): 775-85.

151. Lau LL, Cowling BJ, Fang VJ, et al. Viral shedding and clinical illness in naturally acquired influenza virus infections. J Infect Dis 2010; 201(10): 1509-16.

152. Evans KD, Kline MW. Prolonged influenza A infection responsive to rimantadine therapy in a human immunodeficiency virus-infected child. Pediatr Infect Dis J 1995; 14(4): 332-4.

153. Englund JA, Champlin RE, Wyde PR, et al. Common emergence of amantadine- and rimantadine-resistant influenza A viruses in symptomatic immunocompromised adults. Clin Infect Dis 1998; 26(6): 1418-24.

154. Leung NH, Xu C, Ip DK, Cowling BJ. Review Article: The Fraction of Influenza Virus Infections That Are Asymptomatic: A Systematic Review and Meta-analysis. Epidemiology 2015; 26(6): 862-72.

155. World Health Organization Writing G, Bell D, Nicoll A, et al. Non-pharmaceutical interventions for pandemic influenza, national and community measures. Emerg Infect Dis 2006; 12(1): 88-94.

156. Eccles R. Understanding the symptoms of the common cold and influenza. Lancet Infect Dis 2005; 5(11): 718-25.

157. Ip DK, Lau LL, Leung NH, et al. Viral Shedding and Transmission Potential of Asymptomatic and Paucisymptomatic Influenza Virus Infections in the Community. Clin Infect Dis 2017; 64(6): 736-42.

158. Salgado CD, Farr BM, Hall KK, Hayden FG. Influenza in the acute hospital setting. Lancet Infect Dis 2002; 2(3): 145-55.

159. Mossad SB, Deshpande A, Schramm S, Liu X, Rothberg MB. Working Despite Having Influenza-Like Illness: Results of An Anonymous Survey of Healthcare Providers Who Care for Transplant Recipients. Infect Control Hosp Epidemiol 2017; 38(8): 966-9.

160. Jena AB, Baldwin DC, Jr., Daugherty SR, Meltzer DO, Arora VM. Presenteeism among resident physicians. JAMA 2010; 304(11): 1166-8.

161. Casalegno JS, Eibach D, Valette M, et al. Performance of influenza case definitions for influenza community surveillance: based on the French influenza surveillance network GROG, 2009-2014. Euro Surveill 2017; 22(14).

162. Ferdinands JM, Gaglani M, Martin ET, et al. Prevention of Influenza Hospitalization Among Adults in the United States, 2015-2016: Results From the US Hospitalized Adult Influenza Vaccine Effectiveness Network (HAIVEN). J Infect Dis 2019; 220(8): 1265-75.

163. Norman DC, Yoshikawa TT. Fever in the elderly. Infect Dis Clin North Am 1996; 10(1): 93-9.

164. Ridgway JP, Bartlett AH, Garcia-Houchins S, et al. Influenza among afebrile and vaccinated healthcare workers. Clin Infect Dis 2015; 60(11): 1591-5.

165. Brankston G, Gitterman L, Hirji Z, Lemieux C, Gardam M. Transmission of influenza A in human beings. Lancet Infect Dis 2007; 7(4): 257-65.

166. Bischoff WE, Swett K, Leng I, Peters TR. Exposure to influenza virus aerosols during routine patient care. J Infect Dis 2013; 207(7): 1037-46.

167. Lindsley WG, Pearce TA, Hudnall JB, et al. Quantity and size distribution of cough-generated aerosol particles produced by influenza patients during and after illness. J Occup Environ Hyg 2012; 9(7): 443-9.

168. Fernstrom A, Goldblatt M. Aerobiology and its role in the transmission of infectious diseases. J Pathog 2013; 2013: 493960.

169. Koster F, Gouveia K, Zhou Y, et al. Exhaled aerosol transmission of pandemic and seasonal H1N1 influenza viruses in the ferret. PLoS One 2012; 7(4): e33118.

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

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

172. Loeb M, Dafoe N, Mahony J, et al. Surgical mask vs N95 respirator for preventing influenza among health care workers: a randomized trial. JAMA 2009; 302(17): 1865-71.

173. Johnson DF, Druce JD, Birch C, Grayson ML. A quantitative assessment of the efficacy of surgical and N95 masks to filter influenza virus in patients with acute influenza infection. Clin Infect Dis 2009; 49(2): 275-7.

174. Boyce JM, Pittet D, Healthcare Infection Control Practices Advisory C, Force HSAIHHT. Guideline for Hand Hygiene in Health-Care Settings. Recommendations of the Healthcare Infection Control Practices Advisory Committee and the HIPAC/SHEA/APIC/IDSA Hand Hygiene Task Force. Am J Infect Control 2002; 30(8): S1-46.

175. Creedon SA. Healthcare workers' hand decontamination practices: compliance with recommended guidelines. J Adv Nurs 2005; 51(3): 208-16.

176. Haas JP, Larson EL. Measurement of compliance with hand hygiene. J Hosp Infect 2007; 66(1): 6-14.

177. Chen LF, Carriker C, Staheli R, et al. Observing and improving hand hygiene compliance: implementation and refinement of an electronic-assisted direct-observer hand hygiene audit program. Infect Control Hosp Epidemiol 2013; 34(2): 207-10.

178. Sasaki S, Sullivan M, Narvaez CF, et al. Limited efficacy of inactivated influenza vaccine in elderly individuals is associated with decreased production of vaccine-specific antibodies. J Clin Invest 2011; 121(8): 3109-19.

179. Bowles SK, Lee W, Simor AE, et al. Use of oseltamivir during influenza outbreaks in Ontario nursing homes, 1999-2000. J Am Geriatr Soc 2002; 50(4): 608-16.

180. Edmond MB. Mandatory Flu Vaccine for Healthcare Workers: Not Worthwhile. Open Forum Infect Dis 2019; 6(4): ofy214.

181. Centers for Disease C. Suspected nosocomial influenza cases in an intensive care unit. MMWR Morb Mortal Wkly Rep 1988; 37(1): 3-4, 9.

182. Weinstock DM, Eagan J, Malak SA, et al. Control of influenza A on a bone marrow transplant unit. Infect Control Hosp Epidemiol 2000; 21(11): 730-2.

183. Hota S, McGeer A. Antivirals and the control of influenza outbreaks. Clin Infect Dis 2007; 45(10): 1362-8.

184. Parkash N, Beckingham W, Andersson P, Kelly P, Senanayake S, Coatsworth N. Hospital-acquired influenza in an Australian tertiary Centre 2017: a surveillance based study. BMC Pulm Med 2019; 19(1): 79.

185. Mermel LA. Preventing the spread of influenza A H1N1 2009 to health-care workers. Lancet Infect Dis 2009; 9(12): 723-4.

186. Harris AD, Lautenbach E, Perencevich E. A systematic review of quasi-experimental study designs in the fields of infection control and antibiotic resistance. Clin Infect Dis 2005; 41(1): 77-82.

187. Klinkenberg D, Fraser C, Heesterbeek H. The effectiveness of contact tracing in emerging epidemics. PLoS One 2006; 1: e12.

188. Stone SP, Cooper BS, Kibbler CC, et al. The ORION statement: guidelines for transparent reporting of outbreak reports and intervention studies of nosocomial infection. J Antimicrob Chemother 2007; 59(5): 833-40.

189. Wieland K, Chhatwal P, Vonberg RP. Outbreak reporting a decade after ORION: where do we stand? Lancet Infect Dis 2017; 17(5): 476.

190. Roy S, Hartley J, Dunn H, Williams R, Williams CA, Breuer J. Whole-genome Sequencing Provides Data for Stratifying Infection Prevention and Control Management of Nosocomial Influenza A. Clin Infect Dis 2019.

191. Robinson ER, Walker TM, Pallen MJ. Genomics and outbreak investigation: from sequence to consequence. Genome Med 2013; 5(4): 36.

192. Armstrong GL, MacCannell DR, Taylor J, et al. Pathogen Genomics in Public Health. N Engl J Med 2019; 381(26): 2569-80.

193. Meinel DM, Heinzinger S, Eberle U, Ackermann N, Schonberger K, Sing A. Whole genome sequencing identifies influenza A H3N2 transmission and offers superior resolution to classical typing methods. Infection 2018; 46(1): 69-76.

194. Blackburn RM, Frampton D, Smith CM, et al. Nosocomial transmission of influenza: A retrospective cross-sectional study using next generation sequencing at a hospital in England (2012-2014). Influenza Other Respir Viruses 2019; 13(6): 556-63.

195. Gardy JL, Johnston JC, Ho Sui SJ, et al. Whole-genome sequencing and social-network analysis of a tuberculosis outbreak. N Engl J Med 2011; 364(8): 730-9.

196. Grad YH, Lipsitch M. Epidemiologic data and pathogen genome sequences: a powerful synergy for public health. Genome Biol 2014; 15(11): 538.

197. Eurostat. Health care facilities/hospital beds. Available at: https://ec.europa.eu/eurostat/web/products-datasets/-/tps00046.